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REPORTS

OF THE

COMMISSION

APPOINTED BY

THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,

FOR THE INVESTIGATION OF

MEDITERRANEAN FEVER,

UNDER THE SUPERVISION OF AN

ADVISORY COMMITTEE

OF

THE ROYAL SOCIETY.

PART III.

LONDON:
HARRISON AND SONS, ST. MARTIN'S LANE,
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INTRODUCTION.

In the Introduction to Part I, the history of this investigation into the causation and prevention of Mediterranean Fever was given from its commencement until the end of the summer of 1904.

Major W. H. Horrocks, R.A.M.C., and Dr. R. W. Johnstone, Local Government Board, left Malta at the end of September and arrived in England on October 8, 1904.

The results of the work of the Commission during the summer of 1904 are published in Parts I and II.

At a meeting of the Sub-Committee, held on November 17, 1904, it was decided that Staff-Surgeon E. A. Shaw, R.N., Dr. T. Zammit, Board of Health, Malta, and Captain J. Crawford Kennedy, R.A.M.C., should continue the work during the coming winter, and that Major Horrocks and Dr. Johnstone be asked to return to Malta at the beginning of the following fever season. As Captain Kennedy had been struck off all military duty and was devoting his whole time to the work of the investigation, he was made a member of the Commission.

Major Horrocks returned to Malta about the end of May, 1905, but Dr. Johnstone was unable to take up the work again this summer. Lieut.-Colonel A. M. Davies, R.A.M.C., was therefore appointed a member of the Commission in his place and arrived in Malta on May 28, 1905.

Colonel Bruce, C.B., F.R.S., R.A.M.C., the Chairman of the Sub-Committee, left England on May 19, 1905, and proceeded to Malta, where he met the members of the Commission. Staff-Surgeon Shaw and Captain Kennedy handed to him the papers which form part of the present volume. Dr. Zammit informed him that on account of the pressure of other duties he had been able to do but little work for the Commission, but that he would now be able to devote his whole time to it. He communicated some notes on the feeding of goats with *Micrococcus melitensis*, which seemed to show that the goat is to some extent susceptible to Mediterranean Fever. The following experiments were made by Dr. Zammit:—

Experiment 1.—*White Goat.*

To note the effect of feeding goats on material containing *Micrococcus melitensis.*

1904—

Sept. 15. Examined blood for agglutination. Negative.

„ 18. Fed this goat, adding the contents of a culture of *M. melitensis* on agar to its food.

Dec. 3. Blood has reacted in dilutions of 1 in 20 to 1 in 100, but the temperature curve shows no rise. Fed again in the same way.

„ 23. Blood reacts 1 in 300.

1905—
Apr. 29. Blood reacts 1 in 100. Goat still alive.

Experiment 2.—*Red Goat.*

1904—
Dec. 3. No blood reaction. Fed one tube agar.
„ 5. Fed again.
„ 15. No blood reaction.
„ 23. Blood reacts 1 in 20; 1 in 50 after half-an-hour.
1905—
Apr. 29. Blood reacts 1 in 50.

Dr. Zammit informed the Chairman that he considered goats to be susceptible to Mediterranean Fever, and that the disease is spread to human beings by goats. A temporary laboratory was set up in the Lazaretto buildings, Fort Manoel, to continue the investigation of the disease in goats and also the transference of the disease by mosquitoes.

Colonel Bruce returned to England on June 12, 1905.

On June 23, 1905, Major Horrocks wrote that he had discovered the *M. melitensis* in the milk of an apparently healthy goat, and on the 26th he further wrote that he had already found the *M. melitensis* in the milk of five goats taken from two different herds, and that Dr. Zammit had found it in the blood of one of these goats. Horrocks also said that the milk of the goat fed by Dr. Zammit last September was still crammed with *M. melitensis.* It would therefore appear that the Commission are on the eve of an important and may be far-reaching discovery.

On July 18, 1905, the Chairman received preliminary notes from Major Horrocks, Captain Kennedy, and Dr. Zammit, on the propagation of Malta Fever by means of goats. These are added to the present volume.

These notes show (1) that one or more apparently healthy goats in every herd are excreting *M. melitensis* in their milk and urine; (2) that about 50 per cent. of the goats in Malta react to Mediterranean Fever when examined by the serum agglutination test.

It may be objected that no exact proof exists that the drinking of milk containing *M. melitensis* will give rise to the disease in man. When we take, however, into consideration the results of the feeding and inoculation experiments on monkeys, it may be assumed with safety that the disease is propagated in this way, and that no time should be lost in removing such a grave and insidious danger to the public health.

I. ON A QUANTITATIVE BACTERIOLOGICAL EXAMINATION OF THE BLOOD OF 103 MEDITERRANEAN FEVER PATIENTS.

By Staff-Surgeon E. A. Shaw, R.N., Member Mediterranean Fever Commission, Malta.

Blood.

In my September Report I gave the results of the examination of the peripheral blood of 51 Malta Fever patients for the *Micrococcus melitensis* (hereafter referred to as *M. melitensis*). I now give briefly the results of a further series of 52 such cases similarly examined, making a total of 103 cases examined. If any points seem inadequately explained a reference to the first Report will elucidate matters.

Method.—Bend of elbow prepared as for a surgical operation, carbolic acid being the disinfectant used, a pad of lint soaked in 1 in 20 of this being kept on site of intended puncture till the latter was made. Five c.c. of blood drawn off from median basilic vein in graduated sterile serum syringe; 3 c.c. of this placed in a flask containing 60 c.c. of peptone broth, 1 c.c. into a tube containing 19 c.c. of peptone broth and 1 c.c. into a second tube also containing 19 c.c. of broth, and all these well shaken. The flask containing 3 c.c. of blood and one of the tubes containing 1 c.c. of blood were incubated intact. The second tube containing a mixture of 19 c.c. of broth and 1 c.c. of blood was treated as follows: half of its contents were removed with a 10 c.c. sterile pipette and it was then put aside, now containing $\frac{1}{2}$ c.c. of blood and $9\frac{1}{2}$ c.c. of broth, to be incubated; the contents of the 10 c.c. pipette were then added to a 10 c.c. broth-tube, which was well shaken, and now contained 20 c.c. of fluid, $\frac{1}{2}$ c.c. of which was blood; 10 c.c. of this mixture was removed with the 10 c.c. pipette, leaving it containing $\frac{1}{4}$ c.c. of blood, and it was put aside to be incubated; the contents of the pipette were added to another 10 c.c. broth-tube, which in its turn was left with $\frac{1}{8}$ c.c. of blood, and so on, halving the quantity of blood each time till the following series was obtained: flask containing 3 c.c. of blood, tubes containing 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$, and $\frac{1}{512}$ c.c. blood duly numbered and dated, these were then placed in the incubator at 37°, being taken out daily and well shaken to facilitate distribution of the possible *M. melitensis* throughout the medium. At the end of a week glucose-litmus-agar slopes were inoculated by means of a loop from the 3 c.c. flask, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$ c.c. tubes, these duly labelled and all returned to the incubator. At the expiration of another two days these slopes were examined and those which exhibited growth were put aside to be put through the usual tests for *M. melitensis* (see September Report), and the others with the original broth-tubes returned to the incubator. At the end of a second period of two days the agar slopes were again examined, those showing growth being dealt with as before, and now, it being 11 days since commencement of incubation, those slopes which showed no growth were reinoculated plentifully from the corresponding broth-tubes, and the remainder of the series, the $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$, and $\frac{1}{512}$ c.c. blood broth-tubes, were sub-cultured by means of a large platinum loop on to agar slopes, and all returned to the incubator for a further period of three days, after which all slopes were examined for growth, results recorded, and the examination of that particular specimen of blood terminated.

The foregoing was the general method adopted. It was only slightly departed from in the last 25 cases; in which in order to determine the earliest date at which in these examinations *M. melitensis* made its appearance in the broth, daily sub-cultures were made from the flask containing the 3 c.c. of blood starting from the first day of incubation. As this was never found to be later than the eighth day, and as it will be observed from the foregoing that a total of eleven days' incubation in broth, the same period as observed in the first series of 51 cases, was completed before a blood broth-tube was abandoned as unfruitful, there is obviously an ample margin of safety, and I do not consider that there was any possibility of any fruitful blood broth-tube having been overlooked.

The following table gives the result in a very compressed form; it seems to me unnecessary to write out each blood examination separately. In the remarks which follow the tables, such cases as call for it are discussed in greater detail individually. As all the cases in this series were English and male, nine patients belonging to the navy, and 43 to the army, no column descriptive of nationality and sex has been necessary. In the column "stage of fever," the word "wave" refers to waves of raised temperature. In the column giving patients' temperature for a few days prior to bleeding, the last temperatures are those for the morning and evening of the day blood was drawn, those preceding it being arranged in regular chronological order; the intention being by comparing these, in the cases yielding *M. melitensis*, to ascertain if any relation existed between course of temperature and presence of *M. melitensis* in the blood; these temperatures are given in the form of a fraction, the numerator being the morning and the denominator the evening temperature, the one taken about 8 A.M. the other about 5 P.M. Day of disease, as before, has been calculated from the day the patient first began to feel ill, not from date of admission into hospital. In each case the highest dilution of the patient's blood serum (determined from a portion of the blood taken for bacteriological examination), which gave a distinct agglutination reaction in a quarter of an hour under the $\frac{2}{3}$-in. objective of the microscope, has been worked out and is given in the appropriate column as a dilution of 1 in ..., the unit being serum, the other numeral being so many equivalent bulks of "normal" physiological salt solution.

In the last column "recovery of and smallest quantity of blood yielding *M. melitensis*," "Nil" signifies that in this case the result of the examination was negative. The minimal quantity of blood yielding *M. melitensis* is given as in decimals of a cubic centimetre, a preference having been expressed for this mode of presenting it. To facilitate comparison with the first series in which this quantity was expressed as a fraction, I give the equivalent from $\frac{1}{8}$ onwards—

$\frac{1}{8}$ c.c. = 0·1250 c.c. $\frac{1}{16}$ c.c. = 0·0625 c.c. $\frac{1}{32}$ c.c. = 0·0312 c.c.
$\frac{1}{64}$ c.c. = 0·0156 c.c. $\frac{1}{128}$ c.c. = 0·0078 c.c.
$\frac{1}{256}$ c.c. = 0·0039 c.c. $\frac{1}{512}$ c.c. = 0·0019 c.c.

In the chronological table which comes immediately after the foregoing, fractions have had to be resorted to, because when, as in the 17th and 22nd days of the disease, the results of as many as six blood examinations had to be put down in one space, the use of decimals was found to result in an agglomeration of figures in which "definition" was greatly lacking. Here also the foregoing equivalents may be found useful.

No. of case.	Age.	Stage of the fever.	Temperature of patient for few days preceding bleeding.	Day of disease.	Time of bleeding and patient's temperature.	Maximum dilution of patient's blood giving agglutination.	Recovery of and smallest quantity of blood yielding *M. melitensis*.
			° F.				c.c.
52	21	Nearing end of 1st wave	$\frac{99{\cdot}4}{101{\cdot}6}, \frac{98{\cdot}6}{100}, \frac{98{\cdot}4}{98{\cdot}6}, \frac{100}{98{\cdot}6}, \frac{98{\cdot}4}{98{\cdot}4}$	35th	10.40 A.M., 98°·6	1 in 200	0·5
53	20	Middle of 1st wave	$\frac{98{\cdot}0}{98{\cdot}6}, \frac{98{\cdot}2}{99{\cdot}4}, \frac{100}{102}, \frac{99{\cdot}6}{100{\cdot}2}, \frac{98{\cdot}6}{100{\cdot}8}$	19th	10.55 A.M., 99°·2	1 in 500	0·125
54	20	Middle of 1st wave	$\frac{100{\cdot}6}{102{\cdot}2}, \frac{101{\cdot}4}{102{\cdot}6}, \frac{101}{102}, \frac{99}{101}, \frac{98{\cdot}4}{100{\cdot}8}$	23rd	11.5 A.M., 99°4	1 in 2500	Nil
55	24	Still in 1st wave ...	$\frac{98{\cdot}8}{100}, \frac{98{\cdot}6}{100}, \frac{98{\cdot}8}{100{\cdot}4}, \frac{98{\cdot}8}{99{\cdot}8}, \frac{98{\cdot}8}{100{\cdot}4}$	33rd	10.50 A.M., 99°	1 in 200	0·25
56	35	In 2nd wave.........	$\frac{98{\cdot}2}{100{\cdot}6}, \frac{98{\cdot}6}{101{\cdot}6}, \frac{99{\cdot}2}{102{\cdot}2}, \frac{100{\cdot}4}{102{\cdot}8}, \frac{100{\cdot}8}{103}$	116th	11.0 A.M., 101°	1 in 500	Nil
57	19	Middle of 2nd wave	$\frac{100{\cdot}2}{102{\cdot}8}, \frac{100{\cdot}4}{102{\cdot}6}, \frac{100{\cdot}4}{102}, \frac{101{\cdot}4}{102{\cdot}4}, \frac{101}{102{\cdot}4}$	96th	11.10 A.M., 101°·2	1 in 2000	0·25
58	24	In 1st wave	$\frac{101{\cdot}6}{100{\cdot}2}, \frac{100{\cdot}6}{100{\cdot}8}, \frac{100{\cdot}6}{101{\cdot}2}, \frac{100}{101}, \frac{99{\cdot}6}{102}$	17th	10.50 A.M., 100°·4	1 in 100	0·0312
59	22	In 2nd wave.........	$\frac{99{\cdot}2}{102}, \frac{99{\cdot}6}{103{\cdot}6}, \frac{101{\cdot}6}{103{\cdot}2}, \frac{99{\cdot}6}{103{\cdot}4}, \frac{99{\cdot}2}{102{\cdot}8}$	51st	11.5 A.M., 100°·6	1 in 1000	2·00
60	29	In 2nd wave.........	$\frac{101{\cdot}4}{102{\cdot}6}, \frac{100{\cdot}4}{101{\cdot}2}, \frac{100}{100{\cdot}6}, \frac{100}{101{\cdot}8}, \frac{99{\cdot}8}{100{\cdot}6}$	70th	11.0 A.M., 100°·4	1 in 500	0·0312
61	21	In 1st wave	$\frac{101{\cdot}8}{103}, \frac{99{\cdot}4}{100}, \frac{98{\cdot}6}{98{\cdot}6}, \frac{98{\cdot}4}{98{\cdot}2}, \frac{98}{98{\cdot}2}$	9th	11.10 A.M., 98°·6	1 in 2000	Nil
62	27	In 3rd wave	$\frac{98}{98{\cdot}4}, \frac{98{\cdot}2}{100}, \frac{98{\cdot}4}{99{\cdot}6}, \frac{98{\cdot}4}{99}, \frac{98{\cdot}4}{98{\cdot}8}$	90th	10.45 A.M., 98°·6	1 in 500	0·0078

63	22	In 1st wave	$\frac{98{\cdot}4}{100}$, $\frac{98{\cdot}2}{99{\cdot}2}$, $\frac{98{\cdot}4}{98{\cdot}2}$, $\frac{98{\cdot}6}{98{\cdot}6}$, $\frac{98{\cdot}2}{99}$	40th	11.0 A.M., 98°·4	1 in 500	1·00
64	20	1st wave not yet ended	$\frac{100}{102}$, $\frac{97{\cdot}8}{101}$, $\frac{98{\cdot}4}{102}$, $\frac{97{\cdot}8}{100{\cdot}8}$, $\frac{98{\cdot}4}{101}$	108th	11.10. A.M., 100°	1 in 40	Nil
65	20	In 1st wave	$\frac{101{\cdot}6}{104{\cdot}2}$, $\frac{103}{104}$, $\frac{100{\cdot}2}{103{\cdot}6}$, $\frac{99{\cdot}2}{100{\cdot}4}$, $\frac{98{\cdot}4}{100{\cdot}6}$	16th	11.10 A.M., 100°·2	1 in 40	Nil
66	28	Still in 1st wave ...	$\frac{101{\cdot}4}{102{\cdot}8}$, $\frac{100{\cdot}4}{104}$, $\frac{101{\cdot}4}{103{\cdot}6}$, $\frac{101{\cdot}6}{104{\cdot}2}$, $\frac{101{\cdot}6}{103}$	65th	11.15 A.M., 102°	1 in 500	0·0078
67	20	In 1st wave	$\frac{98{\cdot}4}{100{\cdot}4}$, $\frac{99{\cdot}4}{100{\cdot}6}$, $\frac{99}{100}$, $\frac{98{\cdot}8}{98}$, $\frac{98}{100{\cdot}2}$	22nd	11.10 A.M., 99°	1 in 1000	0·125
68	28	Had 2 waves in 1st attack, then out of hospital 31 days, now in 1st wave of relapse	$\frac{98}{100{\cdot}8}$, $\frac{98{\cdot}6}{100{\cdot}2}$, $\frac{99{\cdot}4}{100}$, $\frac{100{\cdot}6}{102}$, $\frac{98}{101{\cdot}8}$	116th	11.25 A.M., 98°·6	1 in 500	Nil
69	23	In 1st wave	$\frac{100{\cdot}6}{104}$, $\frac{101{\cdot}2}{103{\cdot}4}$, $\frac{99{\cdot}6}{102}$, $\frac{98{\cdot}2}{99{\cdot}8}$, $\frac{98{\cdot}2}{100}$	27th	11.15 A.M., 99°	1 in 20	Nil
70	41	In 1st wave of a relapse	$\frac{98{\cdot}6}{99}$, $\frac{98{\cdot}2}{99}$, $\frac{98{\cdot}2}{99{\cdot}6}$, $\frac{98{\cdot}6}{100}$, $\frac{98{\cdot}6}{100{\cdot}2}$	158th	11.30 A.M., 99°	1 in 40	0·25
71	20	Still in 1st wave ...	$\frac{102}{104}$, $\frac{101{\cdot}2}{103}$, $\frac{102}{103{\cdot}4}$, $\frac{101{\cdot}6}{103{\cdot}6}$, $\frac{101{\cdot}2}{102{\cdot}6}$	56th	12.15 A.M., 102°	1 in 500	0·5
72	21	In 1st wave	$\frac{98{\cdot}4}{100{\cdot}8}$, $\frac{99{\cdot}4}{101{\cdot}6}$, $\frac{100{\cdot}2}{101}$, $\frac{99{\cdot}2}{100{\cdot}4}$, $\frac{99{\cdot}2}{100{\cdot}6}$	17th	11.20 A.M., 99°·8	1 in 1500	0·0625
73	27	In 1st wave	$\frac{99{\cdot}6}{101{\cdot}6}$, $\frac{99{\cdot}6}{102{\cdot}4}$, $\frac{100{\cdot}4}{102{\cdot}2}$, $\frac{101}{102{\cdot}8}$, $\frac{100{\cdot}4}{102{\cdot}6}$	22nd	11.30 A.M., 101°	1 in 2000	0·5
74	25	In 1st wave	$\frac{101}{103{\cdot}2}$, $\frac{100{\cdot}6}{103{\cdot}6}$, $\frac{101{\cdot}2}{103{\cdot}4}$, $\frac{100{\cdot}4}{103{\cdot}4}$, $\frac{101{\cdot}2}{103}$	18th	11.45 A.M., 102°·1	1 in 2000	0·5
75	24	In 3rd wave	$\frac{98{\cdot}6}{100}$, $\frac{98{\cdot}4}{100}$, $\frac{98{\cdot}6}{100{\cdot}2}$, $\frac{98{\cdot}2}{99{\cdot}8}$, $\frac{100}{103{\cdot}3}$	149th	4.45 P.M., 103° 3	1 in 1200	Nil
76	20	In 3rd wave	$\frac{98{\cdot}4}{101{\cdot}2}$, $\frac{98{\cdot}8}{100}$, $\frac{98{\cdot}4}{101{\cdot}2}$, $\frac{99{\cdot}2}{100{\cdot}6}$, $\frac{98{\cdot}5}{102{\cdot}5}$	120th	5.0 P.M., 102°·5	1 in 800	0·0625
77	21	In 3rd wave	$\frac{98{\cdot}4}{100{\cdot}8}$, $\frac{98}{102{\cdot}4}$, $\frac{98{\cdot}6}{102}$, $\frac{98{\cdot}6}{101}$, $\frac{98{\cdot}4}{99}$	151st	5.10 P.M., 99°	1 in 300	1·00

No. of case.	Age.	Stage of the fever.	Temperature of patient for few days preceding bleeding.	Day of disease.	Time of bleeding and patient's temperature.	Maximum dilution of patient's blood giving agglutination.	Recovery of and smallest quantity of blood yielding *M. melitensis*.
			° F.				c.c.
78	29	In 1st wave	$\frac{98\cdot4}{102\cdot2}$, $\frac{98\cdot4}{102\cdot6}$, $\frac{98}{101\cdot6}$, $\frac{98\cdot4}{103\cdot4}$, $\frac{99\cdot4}{103\cdot6}$	35th	10.45 A.M., 100°	1 in 2600	1·00
79	20	In 1st wave	$\frac{98\cdot2}{99\cdot6}$, $\frac{97\cdot8}{101}$, $\frac{98\cdot4}{100\cdot6}$, $\frac{98}{100}$, $\frac{97\cdot6}{100\cdot4}$	18th	11.0 A.M., 98°·4	1 in 3000	0·125
80	22	In 1st wave	$\frac{98\cdot4}{97\cdot6}$, $\frac{98\cdot4}{97\cdot4}$, $\frac{98}{98}$, $\frac{97\cdot4}{98\cdot4}$, $\frac{97\cdot4}{99}$	17th	11.10 A.M., 98°	1 in 2500	0·5
81	24	In 1st wave	$\frac{100\cdot2}{101}$, $\frac{98\cdot4}{102\cdot4}$, $\frac{99}{99\cdot2}$, $\frac{99}{100}$, $\frac{99}{100\cdot2}$	12th	4.40 P.M., 100°·2	1 in 500	Nil
82	27	In 2nd wave.........	$\frac{99}{102}$, $\frac{99\cdot6}{101}$, $\frac{98\cdot4}{101}$, $\frac{99\cdot8}{102\cdot2}$, $\frac{99\cdot2}{101\cdot6}$	67th	4.55 P.M., 101°·6	1 in 1500	1·00
83	37	In 1st wave	$\frac{100\cdot6}{102\cdot4}$, $\frac{99\cdot4}{101\cdot6}$, $\frac{100\cdot4}{100\cdot6}$, $\frac{99}{100\cdot4}$, $\frac{97\cdot8}{100}$	18th	5.10 P.M., 100°	1 in 3000	0·5
84	22	In 1st wave	$\frac{98\cdot2}{99\cdot4}$, $\frac{98\cdot4}{100\cdot4}$, $\frac{98\cdot4}{101}$, $\frac{98\cdot4}{101\cdot6}$, $\frac{98\cdot4}{101\cdot5}$	16th	5.0 P.M., 101°·5	1 in 2000	5·0
85	21	In 4th wave	$\frac{99}{101}$, $\frac{99\cdot4}{101\cdot2}$, $\frac{99\cdot4}{100}$, $\frac{99}{101}$, $\frac{98\cdot6}{102}$	118th	5.10 P.M., 102°	1 in 1500	0·25
86	26	In 1st wave of a relapse after normal T interval of 6 weeks	$\frac{99\cdot2}{102}$, $\frac{99\cdot4}{101\cdot4}$, $\frac{99\cdot4}{101}$, $\frac{99\cdot6}{102\cdot4}$, $\frac{100\cdot4}{103\cdot2}$	153rd	5.20 P.M., 103°·2	1 in 1600	3·00
87	23	In 1st wave	$\frac{N.}{N.}$, $\frac{97}{N.}$, $\frac{N.}{101}$, $\frac{100\cdot2}{N.}$, $\frac{N.}{97\cdot4}$	12th	10.45 A.M., normal	1 in 200	Nil

88	21	In 1st wave	$\frac{101}{102}, \frac{101{\cdot}4}{100{\cdot}6}, \frac{100{\cdot}4}{101}, \frac{100{\cdot}2}{100{\cdot}4}, \frac{100{\cdot}2}{101{\cdot}6}$	23rd	11.5 A.M., 100°·4	1 in 500	0·0312
89	32	In 1st wave	$\frac{100{\cdot}2}{101{\cdot}8}, \frac{101}{101{\cdot}6}, \frac{100{\cdot}4}{102{\cdot}2}, \frac{100{\cdot}8}{101{\cdot}2}, \frac{100}{101{\cdot}6}$	21st	11.15 A.M., 100°·8	1 in 40	Nil
90	19	In 2nd wave.........	$\frac{98{\cdot}8}{102{\cdot}4}, \frac{98{\cdot}2}{101{\cdot}6}, \frac{98{\cdot}2}{102}, \frac{98{\cdot}6}{101{\cdot}2}, \frac{98{\cdot}4}{100{\cdot}2}$	43rd	4.45 P.M., 100°·2	1 in 3000	5·00
91	42	In 2nd wave.........	$\frac{99{\cdot}4}{100{\cdot}8}, \frac{98{\cdot}2}{100{\cdot}6}, \frac{99{\cdot}4}{102}, \frac{98}{99{\cdot}6}, \frac{98{\cdot}4}{99{\cdot}8}$	62nd	5.0 P.M., 99°·8	1 in 500	Nil
92	22	In 1st wave	$\frac{99}{101{\cdot}4}, \frac{100}{101}, \frac{99{\cdot}8}{101{\cdot}4}, \frac{100}{101{\cdot}2}, \frac{100{\cdot}6}{99{\cdot}8}$	41st	5.15 P.M., 99°8	1 in 1200	0·0156
93	20	In 1st wave	$\frac{100{\cdot}2}{101{\cdot}8}, \frac{99{\cdot}4}{103}, \frac{101}{102{\cdot}8}, \frac{101{\cdot}6}{101{\cdot}4}, \frac{101}{103}$	21st	4.0 P.M., 103°	1 in 2000	1·00
94	29	In 1st wave	$\frac{100}{102{\cdot}8}, \frac{101{\cdot}4}{103}, \frac{100}{102{\cdot}4}, \frac{100}{101{\cdot}6}, \frac{100{\cdot}8}{102{\cdot}8}$	31st	4.15 P.M., 102°·8	1 in 2500	1·00
95	35	In 1st wave	$\frac{100}{98}, \frac{100{\cdot}4}{102}, \frac{99}{101{\cdot}2}, \frac{99{\cdot}2}{100{\cdot}2}, \frac{97{\cdot}2}{100{\cdot}4}$	21st	4.30 P.M., 100°·4	1 in 3000	0·0625
96	36	In 1st wave	$\frac{99{\cdot}8}{101}, \frac{100}{99{\cdot}6}, \frac{100}{102{\cdot}4}, \frac{100{\cdot}2}{102}, \frac{101{\cdot}4}{103}$	19th	4.45 P.M., 103°	1 in 2500	0·0312
97	24	In 1st wave	$\frac{102{\cdot}4}{102{\cdot}2}, \frac{98{\cdot}2}{98{\cdot}4}, \frac{97{\cdot}2}{98}, \frac{97{\cdot}8}{97{\cdot}8}, \frac{98}{97{\cdot}8}$	26th	4.0 P.M., 97°·8	?	Nil
98	31	In 1st wave	$\frac{102{\cdot}4}{103}, \frac{102{\cdot}2}{102{\cdot}6}, \frac{100{\cdot}4}{103}, \frac{102{\cdot}2}{102{\cdot}4}, \frac{101{\cdot}2}{103{\cdot}2}$	21st	4.15 P.M., 103°·2	1 in 2500	0·0312
99	19	In 4th wave	$\frac{98}{100{\cdot}2}, \frac{99{\cdot}4}{101{\cdot}8}, \frac{98{\cdot}6}{101{\cdot}8}, \frac{98{\cdot}6}{100{\cdot}2}, \frac{98{\cdot}6}{100{\cdot}2}$	66th	4.30 P.M., 100°·2	1 in 1000	0·0312
100	25	In 1st wave	$\frac{99{\cdot}8}{102{\cdot}2}, \frac{99{\cdot}2}{102{\cdot}4}, \frac{99}{102{\cdot}2}, \frac{100{\cdot}2}{102{\cdot}5}, \frac{101{\cdot}2}{103{\cdot}5}$	25th	4.45 P.M., 103·5	1 in 1500	0·5
101	25	In 1st wave	$\frac{99{\cdot}2}{101{\cdot}6}, \frac{98{\cdot}6}{101}, \frac{99{\cdot}4}{102}, \frac{99{\cdot}2}{102{\cdot}6}, \frac{100}{102}$	32nd	4.15 P.M., 102°	1 in 2400	0·25
102	30	In 1st wave	$\frac{100{\cdot}4}{102{\cdot}8}, \frac{101{\cdot}2}{102{\cdot}8}, \frac{100}{101{\cdot}8}, \frac{99{\cdot}6}{101{\cdot}4}, \frac{98{\cdot}8}{100}$	22nd	4.30 P.M., 100°	1 in 2000	0·0625
103	26	In 1st wave	$\frac{100{\cdot}4}{102{\cdot}2}, \frac{100\;2}{101{\cdot}6}, \frac{99{\cdot}6}{101{\cdot}8}, \frac{99}{101{\cdot}6}, \frac{101}{103}$	17th	4.45 P.M., 103°	1 in 2200	1·00

Examination of Bloods.

Table showing in chronological order the date of the disease in each of the 103 cases in which blood was taken for bacteriological examination and the result. The word "Nil" means no *M. melitensis* was recovered; the days of disease not represented by a blood examination are shown blank. It will be seen that while many days are blank, others are represented by two to six blood examinations. As stated before, this has been unavoidable; the number of cases willing to submit to venous puncture was too small to admit of selection. The minimal amounts of blood yielding *M. melitensis* in each case are expressed in fractions of a cubic centimetre.

Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.
1		37	$\frac{7}{8}$ c.c.	75	
2		38		76	
3		39		77	
4		40	1 c.c.	78	
5		41	Nil, $\frac{1}{16}$, $\frac{1}{64}$ c.c.	79	
6		42	$\frac{1}{128}$ c.c.	80	
7	$\frac{1}{8}$, $\frac{1}{128}$ c.c.	43	5 c.c.	81	
8	$\frac{1}{64}$ c.c.	44		82	
9	Nil, $\frac{1}{9}$, $\frac{1}{8}$, Nil.	45		83	
10	$\frac{1}{9}$, 1 c.c.	46		84	
11	$\frac{3}{4}$ c.c.	47		85	
12	1, Nil, Nil.	48	Nil.	86	
13	Nil.	49	$\frac{9}{10}$ c.c.	87	
14		50		88	
15	Nil, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{256}$ c.c.	51	2 c.c.	89	
16	Nil, 5 c.c.	52		90	$\frac{1}{128}$ c.c.
17	Nil, Nil, $\frac{1}{32}$, $\frac{1}{16}$, $\frac{1}{2}$, 1 c.c.	53		91	
18	Nil, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{2}$ c.c.	54		92	
19	$\frac{1}{8}$, $\frac{1}{32}$ c.c.	55	$\frac{1}{64}$, $\frac{1}{64}$ c.c.	93	
20		56	$\frac{1}{4}$, $\frac{1}{2}$ c.c.	94	
21	Nil, 1, $\frac{1}{16}$, $\frac{1}{32}$ c.c.	57	Nil.	95	$\frac{1}{9}$ c.c.
22	Nil, Nil, 1, $\frac{1}{8}$, $\frac{1}{2}$, $\frac{1}{16}$ c.c.	58		96	$\frac{1}{4}$ c.c.
23	Nil, $\frac{1}{32}$ c.c.	59		97	
24		60		98	$\frac{1}{2}$ c.c.
25	$\frac{1}{256}$, $\frac{1}{2}$ c.c.	61		101	Nil.
26	$\frac{1}{16}$, Nil.	62	Nil.	108	Nil, Nil.
27	Nil.	63		109	
28	Nil, Nil.	64		110	
29		65	$\frac{1}{128}$ c.c.	116	Nil, Nil.
30	Nil, $\frac{9}{10}$ c.c.	66	$\frac{1}{32}$ c.c.	118	$\frac{1}{4}$ c.c.
31	Nil, $\frac{1}{6}$, 1 c.c.	67	1 c.c.	120	$\frac{1}{16}$ c.c.
32	Nil, $\frac{1}{4}$ c.c.	68		149	Nil.
33	$\frac{1}{4}$ c.c.	69	Nil.	151	1 c.c.
34	$\frac{1}{4}$ c.c.	70	$\frac{1}{32}$ c.c.	153	3 c.c.
35	$\frac{1}{2}$, 1 c.c.	71		158	$\frac{1}{4}$ c.c.
36	1, $\frac{1}{64}$ c.c.	72		240	Nil.
		73			
		74	Nil.		

Remarks.—It will be seen from an inspection of the foregoing tables that at any period of this fever up to the commencement of the 6th month of it (158th day) the causal micro-organism may be found in the blood, and in as small a quantity of blood in the course of the 3rd month as in the 1st month.

The smallest quantity of blood from which in this series of 103 observations it has been isolated has been $\frac{1}{256}$ c.c., practically 4 cub. mm., and that only in two cases.

It is unsafe to assume, as one investigator has done, that in any given case the smallest quantity of blood yielding *M. melitensis* contained only one micrococcus. It would be inexact to express the fact that $\frac{1}{256}$ of a c.c. of blood is the smallest quantity of blood yielding *M. melitensis* in a particular case, in the form that this blood contained 256 micrococci per c.c., and equally inexact to state the fact that 0·1 c.c. of blood incubated in 10 c.c. of broth yielded 31 colonies of *M. melitensis* on an agar slope, in the form that this blood contained 310 micrococci per c.c.; or that 0·25 c.c. of blood spread on the surface of an agar in a Petri dish and incubated, yielded 30 colonies of *M. melitensis* as 120 micrococci per c.c. We do not know whether this micrococcus in the blood is free in the plasma, is phagocyted inside a white blood corpuscle, or is present in both these conditions. If inside a leucocyte, we have yet to learn in what period of time the leucocyte can destroy the vitality of the micrococcus. In various experiments made to ascertain the phagocytic power of fresh normal blood on *M. melitensis*, I have frequently seen as many as 20 and 30 micrococci inside one leucocyte, and it is highly improbable that in combining blood with a nutrient medium, the leucocytes are completely fragmented, and any micrococci they may contain evenly distributed through the medium. For these reasons the somewhat cumbrous method of expressing results as minimal quantity of blood yielding *M. melitensis* has been adhered to as having at least the merit of accuracy.

Minimal Quantity of Blood Yielding M. melitensis.

It will be noticed that the minimal quantity of blood necessary to yield *M. melitensis* in these cases varies within very wide limits, from $\frac{1}{256}$ c.c. (Cases 43 and 47) to 5 c.c. (Cases 84 and 90), that is from approximately 4 cub. mm. to 5000 cub. mm. This surely was to be expected; why should it be constant in a series of patients any more than their agglutinating power on *M. melitensis*, which varies from nil to $\frac{1}{3500}$ or $\frac{1}{6000}$; or, indeed, any other clinical phenomenon ?

Here seems the most appropriate place to discuss a feature I made mention of in my former report under the name of "skipping"; where *M. melitensis* was found in some of the higher blood dilutions, and absent from some of the lower, these having been "skipped" or "jumped." This occurred in two of the first series of 51 cases, and in five of the second series of 52 cases. In Case 59 it was recovered only from the broth-tube containing $\frac{1}{16}$ c.c. of blood, all the other tubes remained sterile; excepting the flask containing 3 c.c. of blood here as a total of 2 c.c. of blood was incubated, obviously this had to be reported as a recovery of *M. melitensis* from 2 c.c. In Case 63 *M. melitensis* was recovered only from the 1 c.c. and the $\frac{1}{16}$ c.c. tubes, the others remaining sterile; it is reported as a recovery from 1 c.c. In Case 77 *M. melitensis* was found only in 3 c.c., 1 c.c., and $\frac{1}{16}$ c.c. tubes; it is reported as a recovery from 1 c.c. In Case 84, *M. melitensis* was found only in the $\frac{1}{4}$ c.c. tube, the 3 c.c., 1 c.c., $\frac{1}{2}$ and $\frac{1}{8}$ to $\frac{1}{512}$ c.c. tubes remaining sterile; it is reported as a recovery from 5 c.c. In Case 90, *M. melitensis* was found only in the $\frac{1}{4}$ c.c. tube, the others, 3 c.c. to $\frac{1}{512}$ c.c., remaining sterile; it is reported as a recovery also from 5 c.c.

This phenomenon must, I think, be interpreted as resulting from the small quantity of *M. melitensis* in the circulating blood; in these cases it would seem there was not enough to supply the tubes found sterile, and it would be a matter of chance into which tube the small apparently indivisible amount of *M. melitensis* got.

In no case has the minimal quantity of blood experimented with, $\frac{1}{512}$ c.c. (about 2 cub. mm.) ever yielded *M. melitensis*; in only two cases out of the 103 has the minimal quantity of blood been so small as $\frac{1}{256}$ c.c. (4 cub. mm.). This has a most important bearing on the question of the possibility of the transmission of infection by biting insects such as mosquitoes, which is still *sub judice.*

It is a larger quantity of blood than any biting insect to be found in Malta can contain. Again, with the possible exception of plague, no known bacterial disease has yet been proved to be thus conveyed; this mode of conveyance of infection would appear to be confined to protozoal diseases, and Schandinn's recent work on blood parasites,* in which he demonstrates that in the gnat the "indifferent" spirochætes are so small as only to be visible when a number have agglomerated, and that they can pass through a Chamberland filter, tends to place yellow fever, hitherto considered doubtful in this regard, amongst the protozoal diseases.

Is there any Relation between Temperature of the Patient and the Presence of M. melitensis *in his Blood?*

Taking first the temperatures for the few days preceding and including the date of the abstraction of blood, and grouping these in a tabular form together with the results of the blood examinations, we get the following table :—

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* "Generations und Wirtswechsel bei Trypanosoma und Spirochæte," 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' Band 20, Heft 3, 1904.

Course of patient's temperature.	No. of cases.	No recovery of *M. melitensis.*	Recovery of *M. melitensis.*	Minimal quantities of blood yielding *M. melitensis* where recovered.
				c.c.
Steady between—				
98° and 101°	20	7	13	1, $\frac{9}{10}$, $\frac{7}{8}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$
99° and 102°	22	8	14	1, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{9}$, $\frac{1}{9}$, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{32}$, $\frac{1}{64}$
100° and 103°	20	4	16	3, 2, 1, 1, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$
101° and 104°	12	3	9	$\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{9}$, $\frac{1}{16}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{128}$, $\frac{1}{128}$, $\frac{1}{256}$
102° and 105°	1	—	1	$\frac{3}{4}$
Ascending	7	1	6	5, 1, 1, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{32}$
Descending	14	6	8	5, 1, $\frac{9}{10}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$
Steady about normal	7	5	2	$\frac{1}{2}$, $\frac{1}{2}$
Total cases ...	103	34	69	

Next grouping the cases according to the patient's temperature at the time blood was abstracted, we get:—

Patient's temperature.	No. of cases.	No recovery of *M. melitensis.*	Recovery of *M. melitensis.*	Minimal quantities of blood yielding *M. melitensis* where recovered.
				c.c.
97° to 97°·9...	1	1		
98° to 98°·9...	20	10	10	1, $\frac{9}{10}$, $\frac{9}{10}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{128}$
99° to 99°·9...	21	9	12	1, 1, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{9}$, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{64}$, $\frac{1}{64}$, $\frac{1}{64}$, $\frac{1}{256}$
100° to 100°·9...	22	7	15	5, 2, 1, $\frac{7}{8}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{32}$, $\frac{1}{32}$, $\frac{1}{32}$, $\frac{1}{128}$
101° to 101°·9...	13	5	8	5, 1, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{256}$
102° to 102°·9...	15	1	14	1, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{9}$, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{128}$
103° to 103°·9...	11	1	10	5, 3, 1, 1, $\frac{3}{4}$, $\frac{1}{4}$, $\frac{1}{9}$, $\frac{1}{32}$, $\frac{1}{32}$, $\frac{1}{64}$
Total cases ...	103	34	69	

It will be seen that both these tables show a distinct relation between the presence of *M. melitensis* in the peripheral blood and the patient's temperature; the first showing an increasing ratio of recoveries of *M. melitensis* with the higher temperatures; the second similarly; but no such relationship is visible between temperature and minimal quantity of blood containing *M. melitensis*.

Is there any Relation between the Agglutinating Power of Blood of Mediterranean Fever Cases and the Presence of M. melitensis *therein?*

When commencing the summer examination of these bloods in June, 1904, to corroborate the diagnosis of Mediterranean Fever arrived at by the medical officer in charge of the case, I invariably examined some of the blood drawn for agglutination reaction on *M. melitensis*, and the diagnosis of the cases selected and given is as certain as it can be. After doing a few cases, in was felt it would be of interest to ascertain what, if any, relation existed between high or low agglutinative power and the presence of *M. melitensis* in the blood. It was accordingly necessary to fix on an arbitrary standard to which all recorded agglutination reactions in the series should conform, in no matter what dilution of serum. For the purpose of this work it was therefore laid down that no agglutination reaction would be recorded unless visible under the $\frac{2}{3}$-in. objective of the microscope 15 minutes after contact between diluted serum and emulsion of *M. melitensis* in normal salt solution. The dilutions of the serum were made with a mercury calibrated 5 cub. mm. pipette graduated in $\frac{1}{2}$ cub. mm., the various dilutions and the *M. melitensis* emulsion brought together on a glass slide, which was put in a moist chamber for 15 minutes and then examined under the microscope side by side with a control: and the highest dilution in which agglutination had by then occurred was recorded as the "maximum dilution of patient's blood giving agglutination reaction." It will thus be seen that all these are strictly comparable for both series. This was done for 89 cases out of the 103. It is usual to express a positive agglutination reaction for *M. melitensis* as 1 in "*n*," meaning that 1 bulk of serum in "*n*" bulks of normal saline effects agglutination: this may be expressed as a fraction $\frac{1}{n}$, or again one may say that the agglutinating power of a given patient's serum is "*n*." In the following table in the column "Agglutinating power," the numbers given mean that one bulk of serum diluted with the corresponding number of bulks of normal saline has sufficed to produce agglutination under the conditions already specified; in the column headed "No *M. melitensis*" is placed the number of cases which did not yield *M. melitensis;* in the column headed "Recovery of *M. melitensis*" is placed the number of cases yielding *M. melitensis*, and in the last column are placed the minimal quantities of blood yielding *M. melitensis* for the specified agglutinating power.

Here one can trace no relation between the amount of agglutinating power and the presence or absence of *M. melitensis* in the blood, but there is some indication of a relationship between agglutinating power and minimal quantity of blood yielding *M. melitensis*, which might be tentatively put as follows:—The higher the agglutinating power of a

Agglutinating power.	No *M. melitensis.*	Recovery of *M. melitensis.*	Minimal quantity of blood yielding *M. melitensis* where recovered.	Agglutinating power.	No *M. melitensis.*	Recovery of *M. melitensis.*	Minimal quantity of blood yielding *M. melitensis* where recovered.
			c.c.				c.c.
1 in 20	1			1 in 1080	2		
40	5	3	$\frac{1}{4}$, $\frac{1}{9}$, $\frac{1}{128}$	1200	2	1	$\frac{1}{64}$
100	—	1	$\frac{1}{32}$	1400	1	2	1, 1
200	1	3	$\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{64}$	1500	—	4	5, 1, $\frac{1}{4}$, $\frac{1}{16}$
300	—	1	1	1600	—	2	3, $\frac{1}{4}$
360	—	2	$\frac{3}{4}$, $\frac{1}{6}$	1800	3		
400	—	1	$\frac{1}{64}$	2000	1	7	5, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{16}$
500	5	8	1, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{32}$, $\frac{1}{128}$, $\frac{1}{128}$	2200	—	1	1
600	—	4	1, $\frac{1}{16}$, $\frac{1}{64}$, $\frac{1}{128}$	2400	—	1	$\frac{1}{4}$
640	—	1	$\frac{1}{8}$	2500	1	4	1, $\frac{1}{2}$, $\frac{1}{32}$, $\frac{1}{32}$
800	1	5	$\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{256}$	2600	—	1	1
1000	2	8	2, 1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{256}$	3000	—	4	5, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{16}$

blood during the fever, the larger is the minimal quantity of blood yielding *M. melitensis* likely to be; whence one might be tempted to deduce a correlation between high agglutinating power and high resistance to *M. melitensis* invasion on the part of the patient.

For how long is it Necessary to Incubate in Broth Patients' Blood containing M. melitensis *before its Presence can be Demonstrated?*

To obtain an answer to this question, the broth-tube containing the largest quantity of blood was, in 25 cases, used to inoculate, on each of eight successive days after its abstraction, an agar slope which was dated and incubated at 37° C. Seven of these cases failed to yield *M. melitensis*, in the others it made its appearance variably on the slope inoculated 3, 4, 5, 6, 7, or 8 days after commencement of the incubation of the blood in broth as follows:—

Number of days' incubation.	Number of recoveries.	Minimal amounts of blood ultimately yielding *M. melitensis*.
		c.c.
3	3	$\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$
4	3	1, $\frac{1}{2}$, $\frac{1}{32}$
5	5	1, 1, $\frac{1}{4}$, $\frac{1}{32}$, $\frac{1}{32}$
6	2	1, 1
7	4	5, 5, $\frac{1}{4}$, $\frac{1}{16}$
8	1	3

The general inference to be drawn from this is that, in general, the smaller the "minimal" amount of blood, the earlier *M. melitensis* makes a demonstrable appearance in the blood. The question is of some interest, because Widal, in his similar work on typhoid fever, postulated the hypothesis of the existence in the blood of typhoid cases in variable amounts of what he called "substances empechantes," which delayed the growth of *B. typhosus* in his nutrient broth according to the amount thereof; *B. typhosus*, in his experience, sometimes appearing after one day's incubation, in others not till after eight days. It will be observed that the foregoing cases yield some little support to the theory; for instance, in one case, where the minimal amount of blood was $\frac{1}{16}$ c.c., *M. melitensis* made its appearance after three days; in another case, where the minimal amount of blood was the same, $\frac{1}{16}$ c.c., not till the 7th day.

Diurnal Variation.

In 58 of the 103 cases blood was drawn in the forenoon; in 34 of these cases *M. melitensis* was present, in 24 it was absent; in 45 cases blood was drawn late in the afternoon; *M. melitensis* was present in 35 cases, absent in 10; a ratio of presence to absence of 7 to 5 in the morning, as against 7 to 2 in the evening; that is *M. melitensis* was 2½ times more likely to be found in blood taken in the late afternoon than in the forenoon; this suggests a correlation between the usually higher temperature of the patient in the afternoon and the presence of *M. melitensis* in his blood.

Summary.

1. *M. melitensis* has been demonstrated to be present in the peripheral blood of 68 per cent. (2 out of 3) of the cases examined in a series of 103 cases.

2. *M. melitensis* exists in the blood in relatively small amount, not having been found in association with a less quantity of blood than 4 cub. mm., and that only in two cases out of 103.

3. The higher the temperature of the cases for a few days before and at the period when blood is abstracted, the more likely is the latter to contain *M. melitensis.*

4. The higher the agglutinating power of a blood during the fever, the larger is the minimal quantity of blood yielding *M. melitensis* likely to be. A correlation is suggested between this and the patient's powers of resistance to *M. melitensis.*

5. The smaller the minimal quantity of blood yielding *M. melitensis*, the earlier is *M. melitensis* likely to be obtained from the nutrient broth in which it is being incubated.

6. *M. melitensis* is more likely to be present in blood abstracted in the late afternoon than in the forenoon. A correlation is suggested between this and usual evening rise of temperature.

7. No definite relation can be established between any chronological stage of the fever and the presence of *M. melitensis* in the blood; it is present both early and late in the disease.

II. ON THE INFECTIVITY OF THE SKIN, BREATH, AND SWEAT OF MEDITERRANEAN FEVER PATIENTS.

By Staff-Surgeon E. A. Shaw, R.N., Member Mediterranean Fever Commission, Malta.

1. *Examination of Skin.*

The epidermis is considerably affected in Mediterranean Fever. Hughes states that "about the fourth week desquamation occurs, being most noticeable on the soles of the feet, where the skin peels off in large flakes, leaving the skin of the feet soft and tender for a considerable time," "during the fourth month towards the end of long attacks or even during early convalescence, the hair falls out extensively." "In long cases the nails have often a grooved longitudinally striated appearance." Consequently, in March of 1904, I set myself to make an attempt to determine if *M. melitensis* is excreted in the cast-off epidermal scales of the skin. After some consideration I determined to put epidermal scrapings from Malta Fever Patients into nutrient broth, incubate this, and then from it to attempt to isolate *M. melitensis* if present.

As the skin is well known to be, even under apparently healthy conditions, largely infected with various organisms, and as any attempt at sterilisation thereof might kill off the somewhat delicate *M. melitensis*, and so baulk the object in view, it was felt that some other method of restricting the presence of organisms which would inevitably overgrow the slow growing *M. melitensis* during the process of isolation would have to be resorted to, and the addition of some antiseptic to the nutrient broth which would to some extent hold back the usual skin organisms, and yet not unduly check the possible *M. melitensis*, seemed the most promising method. Formalin was the antiseptic ultimately decided upon, and a series of preliminary experiments enabled one to determine that broth (made with peptone-Martin, and of an acidity + 5 Eyre's scale) containing 1 in 1000 of sodium formate would, after inoculation with *M. melitensis*, give a good growth thereof in four days; 2 in 1000 delayed growth till sixth day, which was felt to be too long, and less than 1 in 1000 would have defeated the object in view of restraining other organisms. The procedure finally adopted was the following:—

Some of the surface epidermis was removed from each selected Malta Fever patient with a sterilised scalpel by scraping the surface of the arms, chest, thighs, and feet; these scrapings were placed in a numbered, dated, test-tube containing the broth specified, incubated at

37° C. for five days (thus giving one day's margin), then the broth-tube was well agitated, a loopful taken from it and placed in another tube, of same number but new date, containing 10 c.c. sterile broth of the kind specified, this well agitated and mixed, and from this dilution zig-zag stroke inoculations were made on large agar (+ 5 Eyre's scale) slope tubes of same number and new date, the new tubes incubated for five days, the agar slopes examined daily for discrete colonies, which never failed to appear, and all of these resembling *M. melitensis* colonies were subjected to the usual tests; the second broth-tube used to form a similar dilution for third generation in broth and on agar slopes, and these again for Fourth Generation.

A total of 14 patients (the opportunity for obtaining material from whom I owe to the kind courtesy of the officers R.A.M.C. of Valletta and Cottonera hospitals, to whom I beg to tender my warmest thanks), all of which cases were undoubted Mediterranean Fever, ranging from three weeks to three months in duration of disease, were thus examined during April, May, and June, 1904. Every case yielded discrete colonies on the agar slopes, many of them greatly resembling *M. melitensis* colonies at first sight, but proving on further examination to be a white staphylococcus, apparently Welsh's skin staphylococcus, and not one of them turned out to be a colony of *M. melitensis*.

In August the foregoing method was modified as follows, the broth enrichment method being abandoned :—The epidermal scrapings from each patient were thoroughly ground up in 1 c.c. of sterile normal salt solution, one loopful of this was used to plate three successive glucose-litmus-agar Petri dishes; the remaining epidermal emulsion was diluted by the addition of 5 c.c. more normal saline, and the surface of three other similar Petris inoculated by spreading $\frac{1}{4}$ c.c. of this diluted epidermal emulsion over each, and the six plates then incubated at 37° for five days, at the end of which they were carefully examined for possible colonies of *M. melitensis*, and all likely looking ones put through the usual tests. A total of 71 specimens were thus examined. The accompanying table shows the cases and the day of disease on which specimens were taken, the sign × indicating each examination. The first 14 cases are not included, the day of disease not having been sufficiently accurately recorded.

From none of these bacteriological examinations has *M. melitensis* ever been recovered, but in nearly every plate out of the 426 used in this investigation has been found most constantly a Gram staining glucose fermenting white staphylococcus, presumably the same described by Welsh as associated with the skin, and on taking off the covers of the Petri dishes, a faint sour odour very similar to that noticeable on raising the bed-clothes of a feverish rheumatic patient was generally perceptible, suggesting the possibility of "sour sweats" being due to fermentations set up by this organism.

Table showing Cases and Day of Disease on which Skin was examined Bacteriologically.

Patient's Name.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Mayes																														×											
Francis																																			×						
Hagger																																									
Kelly																																							×		
Wilson																																			×						
Jones																																									
Martin																																									
Lawrence																																								×	
Hewett																	×																								
Hurrell												×																													
Rivers										×																															
Shelly															×																										
Kinsella																																									×
Marchant																										×															
Silburn																															×										
Campbell																																									×
Turner																																									
Donovan																			×				×				×			×											
Rentcome																																									×
Smith																																									
Joyce																																×			×			×			×
Ericson									×												×				×										×						
Warren																																									
Anthony																																									
Bolt																																									
Derby																	×				×																				
Dennis																																									
Gane																																									

Table showing Cases and Day of Disease on which Skin was examined Bacteriologically—*continued.*

Patient's Name.	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91
Mayes					×																																				
Francis																																									
Hagger									×																																
Kelly																																									
Wilson																																									
Jones																		×																							
Martin									×	×																															
Lawrence												×																													
Hewett																																									
Hurrell																																									
Rivers																																									
Shelly																																									
Kinsella				×			×				×				×				×		×																				
Marchant											×																														
Silburn																																									
Campbell			×																																						
Turner			×							×				×				×																							
Donovan																																									
Rentcome				×				×			×				×			×			×			×					×												
Smith											×				×			×			×			×					×			×									
Joyce																																									
Ericson																																									
Warren				×			×					×			×																										
Anthony																																									×
Bolt																																				×				×	
Derby																																									
Dennis																												×			×										
Gane									×																																

Animal Experiments with Skin Scrapings.

It was necesssary to see also whether any evidence of the presence of *M. melitensis* in the skin of patients could be obtained by animal experimentation, and accordingly the following were undertaken :—

Experiment I.—Monkey No. 54.

A freshly arrived animal whose temperature and whose blood gave not the slightest indication of reaction to *M. melitensis* was set apart for this experiment.

July 21. Epidermal scrapings from the arms, forearms, and flanks of six Malta Fever patients, all between 28th and 35th day of disease, were taken, ground up in 5 c.c. sterile nutrient broth in a small sterilised mortar with a sterile pestle, and the resulting emulsion injected subcutaneously with a sterile syringe between the animal's shoulders, the intention being to first get general evidence, and later work out details.

July 22. Evening temperature had risen to 104° F.

July 24. Injection not yet showing any indication of absorption; persists as a globular swelling.

July 25. Some indication of commencing suppuration near site of injection. Evening temperature 104° F.

July 27. Commencing suppuration of 25th has now aborted. Injection still persists as a globular swelling.

July 29. Injectional swelling now disappearing.

July 31. No trace of agglutination in a dilution of $\frac{1}{20}$.

August 3. Temperature has remained normal since July 24. Again made emulsion of the skin-scrapings from five patients all between 30th and 45th day of disease, and injected subcutaneously.

August 5. No swelling, but some induration at sight of last injection.

August 7. No trace of agglutination reaction in a dilution of $\frac{1}{10}$. This monkey's box is next to that of Monkey No. 55, which, unknown to me, had been artificially infected with Malta Fever, and contact was possible (see note at end of this experiment).

August 9. Evening temperature to-day 104°, though normal from July 26 to this morning.

August 10. Agglutination reaction is present in a dilution of $\frac{1}{10}$ visible to naked eye, in $\frac{1}{20}$ visible under $\frac{2}{3}$-in. objective.

August 11. Temperature again normal. Again repeated injection of epidermal scrapings from four other cases of Malta Fever, all between 30th and 45th day of disease.

August 15. Agglutination reaction present in a dilution of $\frac{1}{10}$ visible to naked eye, and of $\frac{1}{20}$ visible under $\frac{2}{3}$-in. objective.

August 26. Agglutination reaction present as on August 15.

September 5. Agglutination reaction visible to naked eye in a dilution of $\frac{1}{20}$, and in $\frac{1}{40}$ under $\frac{2}{3}$-in. objective.

September 28. Agglutination reaction as on September 5.

October 1. Agglutination reaction as on September 5. There has been no rise of temperature since August 9. Monkey killed with chloroform, an aseptic *post-mortem* made, and two broth-tubes and one agar slope inoculated with small cubes of tissue from the spleen, the broth-tubes each receiving a piece of the organ; similarly the liver and kidney, and all incubated. All organs were healthy, and there was no enlargement of the spleen.

October 8. No growth in any slope of October 1. Now inoculated six slopes from the six broth-tubes of October 1.

October 13. No growth in any slopes of October 1 or October 8. Experiment concluded.

Note.—As mentioned above, this monkey had been unwittingly exposed to the possibility of contact infection from Monkey No. 55, but as the animal cannot be safely said to have developed the fever, this does not matter. It had an occasional rise of temperature, but lasting only a day. No *M. melitensis* was recovered *post-mortem*, and an agglutination of $\frac{1}{40}$ alone is insufficient on which to base a diagnosis, and I should consider the development of this reaction due to the injection of *M. melitensis* toxins (contained in the skin). Of the action of toxins in producing the agglutination reaction I give experimental evidence in another section of this Report.

Experiment II.—Monkey No. 68.

During the first weeks of the last experiment the possibility of excretion of *M. melitensis* in the urine became an established fact, and as then the possibility of patients infecting the skin of their flanks with their own urine had to be considered, it was resolved that henceforth only scrapings from the upper arms of patients should be used. Further, the monkey (No. 68) used for this second experiment had his box put in a corner with no neighbour on his left, and the monkey used in the preceding experiment on his right, and as the latter did not develop the fever, and No. 68 was within reach of no other, he must be considered as not having been exposed to the risk of contact infection.

Monkey No. 68 was kept under observation from August 12 to 20; during this period his temperature varied from 100°—103°·2; blood presented no trace of agglutination reaction with *M. melitensis.*

August 20. Epidermal scrapings from upper arms of five patients, all between 30th and 60th day of disease, were emulsfied as before, and injected between shoulders subcutaneously.

August 26. There has been no fever since last note, and to-day there is no trace of agglutination reaction.

August 27. Second injection of arm scrapings from two patients, both in 91st day of disease.

September 2. Third injection of arm scrapings of three patients, all between 60th and 90th day of disease.

September 5. Agglution reaction present in a dilution of $\frac{1}{40}$, not beyond.

September 9. Ill, and off his feed.

September 10. Died between 5 and 7 P.M. In my absence an immediate *post-mortem* was made by Major Horrocks, who notes: "Very emaciated, maggots on skin of face, spleen and kidneys appeared slightly congested, other viscera appeared normal. Made cultures from spleen and kidney."

September 19. Agar slopes inoculated from spleen on 10th have remained sterile. Kidney slopes planted from broth on 14th also sterile; reinoculated these.

September 20. Reinoculated spleen slopes.

September 25. All remain sterile.

Result.—No development of fever, but development of a low ($\frac{1}{40}$) agglutination reaction, probably the result of injection of *M. melitensis* toxins in the epidermal scrapings. No *M. melitensis* recovered *post-mortem.*

Experiment III.—Monkey No. 62.

This experiment was commenced by Major Horrocks, and was turned over by him to me to complete on September 28, just prior to his departure for England. For convenience of comparison, I will briefly recapitulate Major Horrocks' notes.

Monkey No. 62 had had its blood frequently examined during August, and up to commencement of the experiment, and its temperature had been daily recorded. It was absolutely free from any suspicion of Malta Fever.

September 16. Monkey No. 62 received an injection of skin scrapings from arms and axilla of a fever patient, ground up in normal saline solution.

September 21. Blood examined, no agglutination reaction to *M. melitensis.*

September 24. Skin scrapings from same patient again injected.

September 26. Blood again examined. No agglutination reaction to *M. melitensis.*

September 27. Skin scrapings from same patient again injected.

September 30. This morning monkey was too sick to have his temperature taken. Died at 10.30 A.M. *Post-mortem* made at once. All organs seemed healthy. No cause of death discoverable. Inoculated broth-tubes and agar slopes from spleen, kidney, liver and heart's blood and incubated.

October 7. No growth on any slope of September 30. Incubated fresh slopes from broth-tubes of September 30.

October 12. No growth on any slope of October 7. Experiment concluded.

Result.—No development of Malta Fever. No development of agglutination reaction. No recovery of *M. melitensis*, *post-mortem.*

Note.—This experiment differs from the other two preceding it only in the non-appearance of the agglutination reaction; but, as in Experiment I, Monkey No. 54, the first skin injection was given July 21, and the *agglutination reaction* did not appear till August 10, an interval of 21 days, nor in Experiment II, Monkey No. 68, till after an interval of 16 days; nor in Experiment IV, Monkey No. 74, next to be described, till after an interval of 22 days; nor in Experiment V, Monkey No. 65, to be described next but one, till after an interval of 23 days; I think one is entitled to consider that as Monkey No. 62, Experiment III, died 14 days after its first skin injection, an interval shorter than any of those just cited, that there had not been time for the agglutination reaction as observed in the others, to develop.

Experiment IV.—Monkey No. 74.

This experiment, like the last, was commenced by Major Horrocks on September 12, during my absence, and turned over to me on September 28 to complete. Blood had prior to experimentation been frequently examined, but had never exhibited the slightest agglutination reaction with *M. melitensis.* There was never any possibility of contact infection.

September 12. Injection of emulsified skin scrapings from arms and axilla of one Mediterranean Fever patient.

September 17. Blood examined. Serum in a low dilution appeared to have a tendency to agglutinate *M. melitensis.*

September 23. Blood again examined. Serum in a dilution of 1 in 10 showed no sign of agglutinating *M. melitensis* after contact of one hour.

September 25. Second injection of skin scrapings emulsified in normal saline.

September 27. Third injection of skin scrapings.

September 28. Blood examined. No agglutination reaction.

October 3. Fourth injection of skin scrapings.

October 4. Blood gives a distinct agglutination reaction in a dilution of $\frac{1}{40}$ in 15 minutes visible under $\frac{2}{3}$-in. objective.

October 8. Fifth injection of skin scrapings from three patients.

October 11. Blood gives distinct agglutination reaction in a $\frac{1}{80}$ dilution after 15 minutes visible to naked eye.

October 15. Sixth injection of skin scrapings from same three patients.

October 18. Agglutination reaction visible to naked eye in $\frac{1}{80}$ dilution.

October 19. Seventh injection of skin scrapings from three patients.

October 22. Eighth injection of skin scrapings from two patients.

October 25. Agglutination reaction in $\frac{1}{40}$ dilution visible to naked eye and in $\frac{1}{60}$ visible under $\frac{2}{3}$-in. objective.

October 26. Ninth injection of skin scrapings from four patients.

October 28. This morning this monkey was found dead. *Post-mortem.* Stomach much dilated with gas. Organs all apparently healthy, much muscular wasting, abscesses at sites of injections. Broth-tubes and agar slopes inoculated from all organs and incubated.

November 2. Agar slopes of October 28 all sterile. Inoculated fresh agar slopes from broth tubes and incubated.

November 7. Agar slopes of November 2 sterile, as are also those of October 28. Experiment concluded.

Result.—Injection of skin scrapings has not been followed by fever, but has developed an agglutination reaction in the serum in low dilution appearing after an interval of 22 days from date of first injection.

Experiment V.—Monkey No. 65.

This monkey had been bitten in September by supposedly infected mosquitoes, but had never had any fever, nor had its blood, which with the others had been examined as a routine measure, once a week, ever shown any sign of agglutinating power on *M. melitensis.*

October 30. This monkey received an injection of epidermal scrapings from arms of four Mediterranean Fever patients.

November 3. Second injection of skin scrapings from four patients.

November 6. Third injection of skin scrapings from four patients.

November 7. Agglutination reaction = a slight tendency only visible in $\frac{1}{10}$, $\frac{1}{20}$ and $\frac{1}{40}$ dilutions, insufficient to call positive.

November 10. Fourth injection of skin scrapings from arms of four patients.

November 13. Fifth injection of skin scrapings from arms of four patients.

November 15. Agglutination reaction = nil in dilutions of $\frac{1}{10}$, $\frac{1}{20}$, and $\frac{1}{40}$.

November 16. Sixth injection of skin scrapings from arms of four patients.

November 19. Seventh injection of skin scrapings from arms of four patients.

November 22. Agglutination reaction distinct traces in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions only.

November 24. Eighth injection of skin scrapings from arms of four patients.

November 27. Ninth injection of skin scrapings from arms of four patients.

November 28. Agglutination reaction marked in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions.

December 1. Tenth injection of skin scrapings from arms of four patients.

December 4. Eleventh injection of skin scrapings from arms of four patients.

December 5. Agglutination reaction only in $\frac{1}{10}$ dilution visible under $\frac{2}{3}$-in. objective.

December 12. No agglutination reaction.

December 19. No agglutination reaction.

December 26. No agglutination reaction.

January 3. No agglutination reaction.

January 9. No agglutination reaction.

January 16. No agglutination reaction.

January 23. No agglutination reaction.

Experiment concluded. Monkey used for other experiments.

Result.—Infection of skin scrapings has not been followed by fever, but has developed in the blood a low agglutination reaction on *M. melitensis* after an interval of 23 days.

Remarks.—Summarising in tabular form the foregoing five experiments we get the following (see Table, p. 30).

In none of these monkeys can Malta Fever be said to have developed. The appearance of a low agglutination reaction alone is not sufficient on which to base a diagnosis and may, I think, safely be attributed to the presence of *M. melitensis* toxins in the skin scrapings used. It is to be noted that the highest dilution in which it was obtained was $\frac{1}{80}$; this is identical with the highest dilution which the agglutination reaction was obtained in Monkeys 58 and 59, which each received injections of the filtrate from broth in which *M. melitensis* had been cultured (*vide* toxin experiments), and both contrast markedly with the high dilution $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{3000}$, $\frac{1}{3500}$ in which agglutination is quite usually obtainable in monkeys which have received living *M. melitensis* in any experimental manner. The occasional elevations of temperature are attributable to the presence of staphylococci in the skin scrapings used, which had a decided tendency to produce abscess formation.

The point with regard to the toxins was felt to be an important one, and accordingly an effort was made to resolve it by an experiment which was commenced by Major Horrocks on September 22 and continued by me from September 28 to its conclusion on December 26.

Experiment number.	Monkey number.	Different samples of epidermis.	Number of injections.	Date of first injection.	Date of appearance of agglutination reaction.	Number of days required to develop agglutination.	Highest dilution which gave agglutination.	*Post-mortem* examination for *M. melitensis*.
I	54	15	3	July 21	Aug. 10	20 days	$\frac{1}{20}$	Not recovered.
II	68	10	3	Aug. 20	Sept. 5	16 ,,	$\frac{1}{40}$	Not recovered.
III	62	3	3	Sept. 16	Never appeared. Died Sept. 30	...	...	Not recovered.
IV	74	18	9	Sept. 12	Oct. 4	22 days	$\frac{1}{80}$	Not recovered.
V	65	44	11	Oct. 30	Nov. 22	23 ,,	$\frac{1}{20}$	No *post-mortem*.

Experiment VI.

Monkey No. 61A. Had never had any elevation of temperature and had never presented agglutination reaction.

September 22. Skin scrapings from arms and axillæ of two Malta Fever patients were ground up with sterile normal salt solution into a fine emulsion. A sterile Berkefeld candle was fitted to a sterile test-tube, the emulsion filtered, and the filtrate injected subcutaneously into Monkey No. 61A.

September 24. Sweat obtained from three Malta Fever patients, similarly filtered and filtrate injected.

September 26. Blood presented no agglutination reaction.

October 3. Blood presented no agglutination reaction.

October 6. Injected filtered sweat from one patient.

October 8. Skin scrapings from three patients ground up in 15 c.c. sterile salt solution, allowed to macerate at laboratory temperature, 68° F., for two hours, filtered through Berkefeld candle and filtrate injected.

October 10. Blood presented no agglutination reaction.

October 15. Skin scrapings from three patients treated as on October 8 and filtrate injected.

October 17. Blood presented no agglutination reaction.

October 19. Skin scrapings and sweat from four patients treated as on October 8, and filtrate injected.

October 22. Skin scrapings and sweat from three patients treated as on October 8, and filtrate injected.

October 24. Blood presented no agglutination reaction.

October 26. Skin scrapings from four patients, treated as on October 8, and filtrate injected.

October 30. Skin scrapings from four patients treated as on October 8, and filtrate injected.

October 31. No agglutination reaction.

November 3. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 6. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 7. Blood presented no agglutination reaction.

November 10. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 13. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 14. Blood presented no agglutination reaction.

November 16. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 19. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 21. Blood presented no agglutination reaction.

November 24. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 27. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 28. Blood presented no agglutination reaction.

December 1. Skin scrapings from four patients treated as on October 8, and filtrate injected.

December 4. Skin scrapings from four patients treated as on October 8, and filtrate injected.

December 26. Blood has been examined weekly since last note and also to-day and has never presented any agglutination reaction with *M. melitensis.*

Experiment concluded.

Remarks.—This experiment, so far as it goes, would appear to negative the explanation given of the appearance of the agglutination reaction in four out of the five preceding experiments, but it is to be observed that it takes for granted one important point, *i.e.*, that *M melitensis* "toxins" are soluble in normal salt solution. This point is reserved for future experiments, as it does not affect the main inference to be drawn from this series of experiments.

Conclusion.—The active infective agent of Mediterranean Fever is not excreted by the skin.

2. *Examination of Breath.*

With a view to obtaining experimental evidence on this question it was determined to instruct patients to gently blow through sterile broth contained in sterile tubes and examine this broth bacteriologically. Broth tubes were fitted with rubber corks bored with two holes, through one of which was passed a long glass entry tube, bent outside the broth-tube at an obtuse angle of about 150°, length outside broth-tube being about 3 inches, inside dipping beneath surface of broth; through the other hole in the rubber cork was passed a short straight glass air-exit tube lightly plugged with sterile cotton wool 1-inch in length on each side of the rubber cork. The patients whose breath it was desired to examine, were instructed to blow gently down the long entry tube at frequent intervals during one hour, their expired air gently bubbling through the broth. In experiments made before the present series the entry tube outside the broth-tube was fitted with a longish piece of rubber tube and a glass mouth-piece, but it seemed to me that if expired air contained any microbes there was great risk of these being caught by the moist inner surface of the rubber tube, on which a considerable proportion of the water vapour contained in all expired air was found to condense.

First Method.—These broth-tubes on arrival at the laboratory had their rubber corks and glass tubes removed, were replugged with sterile cotton wool, incubated for seven days at 37°, and then one loopful from each distributed over the agar surfaces of two large-sized Petri dishes, which were incubated for five days at 37°, and then examined for individual *M. melitensis* colonies. A total of 86 such breath-tubes were examined in the manner described, but *M. melitensis* never appeared in any plate, though other organisms, not examined in detail, did.

Second Method.—At this stage other experiments on the vitality of *M. melitensis* growing with other organisms (see Section on Vitality of *M. melitensis* outside the Body, p. 43) had shown that *M. melitensis* had not much chance of surviving in a fluid nutrient medium with other microbes. Accordingly from each broth-tube immediately on its arrival at the laboratory, after well shaking, a loopful was taken and distributed over the agar surface of two Petri dishes, the tubes then plugged with sterile cotton wool and both tubes and plates incubated. At the end of five days these "direct" plates were carefully examined for *M. melitensis*, and at the end of seven days' incubation a loopful from each breath broth-tube was distributed over two agar plates and these also examined after five days' incubation for possible *M. melitensis*. One thus had two series of plates, one direct, the other after the incubation of the breath-infected broth. A total of 24 breath-tubes were treated in this manner, but no *M. melitensis* ever appeared on any plate, though the same type of other organisms as before were found.

Third Method.—By this time experiments with *M. melitensis* in association with other organisms had shown that a period of three days was as long as one could expect to recover *M. melitensis* when incubated in broth with other organisms. Accordingly the "direct" series of plates was continued as before, but the breath broth-tubes were only incubated for three days and then plated.

I examined 115 breath broth-tubes in this way, but not in one did I find a single *M. melitensis* colony.

A total of 225 such broth-tubes were examined, and in connection therewith 728 agar Petri plates were prepared and examined, all without result so far as the particular quest involved was concerned. I append a table showing names of patients and day of disease in each case in which breath broth-tubes were prepared, which is indicated in each case by the sign ×. It will be seen that practically the whole period of the disease has been covered.

Table showing Cases and Day of Disease on which Breath was Examined Bacteriologically.

Patient's name.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Anthony																×																															
Rivers																																							×								
Silburn																			×			×						×				×	×											×		×	
Tripp																																	×														
Anderson ...																											×																				
Rentcome ...																				×			×																								
Russell																																			×			×			×						
Marchant ...																		×																													
Donovan		×						×			×	×	×	×	×	×										×										×	×		×	×		×		×			
Kinsella																																						×						×	×	×	×
Campbell ...																																				×		×	×	×	×	×	×	×			
Dinegan																			×																												
Joyce						×	×									×	×		×						×																	×		×	×	×	×
Grimwood ...																×	×		×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×		
Groom																																															
Fletcher										×																																					
Tait										×		×																																			
Smith																																															
Turner.........																																															
Dennis.........																																															
Ericson																×		×																×													
Darby																					×	×	×	×	×	×																					
Walker						×	×	×		×	×																																				

Table showing Cases and Day of Disease on which Breath was Examined Bacteriologically—*continued.*

Patient's name.	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93
Anthony																																														
Rivers																																														
Silburn			×		×	×	×	×	×	×	×	×	×																																	
Tripp																																														
Anderson ...																																														
Rentcome ...																																														
Russell											×		×		×		×		×		×		×																							
Marchant ...																																														
Donovan																																														
Kinsella	×	×	×		×	×		×		×		×	×	×		×		×		×	×	×		×		×																				
Campbell ...																																														
Dinegan																																														
Joyce	×	×	×	×				×																																						
Grimwood ...		×	×	×	×	×	×	×	×	×	×		×	×	×	×		×	×	×	×	×		×	×																					
Groom.........													×		×		×	×			×	×	×	×	×	×	×			×	×	×														
Fletcher																																														
Tait																																														
Smith					×	×	×	×	×	×	×		×		×	×	×	×		×	×	×	×	×		×				×																
Turner.........																		×	×	×	×	×		×		×		×							×	×	×	×				×			×	
Dennis.........										×		×		×		×	×	×	×	×	×	×	×	×	×	×	×	×		×	×															
Ericson																																														
Darby																																														
Walker																																														

In order to further investigate the possibility of the infection of Malta Fever being given off in the breath, animal experimentation was also resorted to; portions of such of the foregoing broth-tubes as presented growth being injected in quantities usually of 10 c.c. into two monkeys. This was commenced in the first monkey, No. 73, by Major Horrocks, September 16, and continued by me from September 28 till this monkey's death on November 1; in the second monkey, No. 43, commenced and concluded by me alone.

Monkey No. 73.—This animal had never been used for any other experiment.

September 15. Blood examined. No reaction to *M. melitensis.*

September 16. Injected contents of broth-tube infected by Malta Fever patient's (Lawrence) breath in which growth had occurred after incubation.

September 21. Similar injection (Silburn).

September 28. Blood examined. No reaction to *M. melitensis.*

October 3. Big abscess at site of former injections. To-day injected a growth in breath broth-tube (Anderson) on opposite side.

October 4. Blood examined. No reaction to *M. melitensis.*

October 6. Injected broth growths from breath of Rentcome and Marchant, 5 c.c. from each.

October 9. Similar injection (Silburn).

October 11. Blood examined. Agglutination reaction with *M. melitensis* in $\frac{1}{40}$ dilution under $\frac{1}{6}$-in. objective.

October 12. Injected broth growths from breaths of Campbell, Joyce and Silburn, 3 c.c. each.

September 16. Injected broth growths from breaths of Campbell, Grimwood and Kinsella, 3 c.c. each.

October 18. Blood presents agglutination reaction in a dilution of $\frac{1}{100}$ visible to naked eye.

October 19. Injected broth growth from breaths of Campbell and Grimwood, 5 c.c. of each.

October 23. Injected broth growths from breaths of Campbell, Kinsella, and Joyce, 3 c.c. of each.

October 25. Agglutination reaction in a dilution of $\frac{1}{100}$ visible to naked eye.

October 26. Injected broth growths from breaths of Fletcher, Groom, Russell and Tait, $2\frac{1}{2}$ c.c. from each.

October 29. Monkey ill. Considerable diarrhœa and wasting.

November 1. Monkey obviously dying. Euthanasia cum chloroform. *Post-mortem.*—Much wasting, no obvious cause of death, organs all apparently healthy, gas in intestines. Agar slopes and broth-tubes inoculated from all organs and incubated.

November 6. Agar slopes of November 1 all sterile. Inoculated fresh slopes from broth-tubes of November 1.

November 11. Agar slopes of November 6 also sterile.

Experiment concluded.

Monkey No. 43.—This animal, in July, on the 16th and 18th, had received injections of the filtrate through filter paper of supposedly infected soil macerated in sterile water, but had never developed Malta Fever, nor had its blood ever reacted to *M. melitensis* in any dilution whatever, though frequently examined.

October 3, 10, 17, 24. No trace of agglutination reaction in dilutions of $\frac{1}{10}$, $\frac{1}{20}$, or $\frac{1}{40}$.

October 27. Injected broth growth from breaths of Grimwood, Joyce and Silburn, 3 c.c. from each.

October 31. Injected broth growth from breaths of Donovan and Silburn, 5 c.c. from each. No agglutination reaction in $\frac{1}{10}$, $\frac{1}{20}$ or $\frac{1}{40}$ dilutions.

November 4. Injected broth growth from breaths of Groom and Silburn, 5 c.c. each.

November 7. A doubtful tendency to agglutination in $\frac{1}{10}$, $\frac{1}{20}$ dilution under $\frac{1}{6}$-in. objective. Abscess at the site of last injection but one.

November 8. Injected broth growth from breaths of Turner and Kinsella, 5 c.c. each.

November 12. Monkey somewhat ailing.

November 14. No agglutination reaction.

November 17. Injected broth growth from breath of Grimwood, 10 c.c.

November 21. Injected broth growth from breaths of Dennis and Grimwood, 5 c.c. each.

November 21. No agglutination reaction.

November 28. Slight agglutination reaction in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions under $\frac{2}{3}$-in. objective.

December 1. Injected broth growth from breaths of Darby and Walker, 5 c.c. each.

December 4. Injected broth growth from breaths of Darby, Walker, and Turner, 5 c.c. each.

December 5. Tendency to agglutination in $\frac{1}{10}$ dilution.

December 12. No agglutination reaction. Very weak, thin and emaciated; has been seedy for some days.

December 15. Dying. Gave chloroform. *Post-mortem*, found pneumonia and pericarditis left side. Inoculated slopes and broth-tubes from all organs.

December 23. No *M. melitensis* recovered *post-mortem*, but a glucose fermenting + Gram-staining coccus was obtained from spleen, liver and kidney; nothing from heart's blood and lungs.

Remarks.—There is to be noticed in both these animal experiments the development of a low agglutination reaction, and here as in the skin experiments I should attribute this to the ingestion of *M. melitensis* toxins, as I consider it practically certain that in breathing out through the broth-tubes, a certain amount of saliva trickled down the long entry tube and so into the broth; the possibility of this was considered at the time and efforts were made to arrange some method of passing breath containing possibly *M. melitensis*, while excluding saliva; but none free from objection was found, hence it was decided to proceed as described, and if *M. melitensis* were obtained, to examine the saliva of patients independently for this micro-organism. In neither monkey could it be said that Mediterranean Fever was developed. The agglutination reaction developed was much too low, the occasional rises of temperature observed were attributable to abscess formation, or to other micro-organisms, not *M. melitensis*, contained in the broth growths which were injected. In both cases temperatures were taken morning and evening all through the experiments.

3. *Examination of Sweat.*

Critical sweats are a not infrequent and quite characteristic feature of Mediterranean Fever, and it has been often felt that it was not impossible that the *Micrococcus melitensis* might be passed out of the body in this secretion. To determine this exhaustively, I made a bacteriological examination of 251 specimens of sweat obtained from patients in the Military Hospital at Valetta. The method adopted was varied from time to time, as will be described.

First Method.—A skin surface of forearm washed with spirit soap, then ether, a carbolic pad 1 in 40 kept on 12 hours, then a circle of sterilised (dry, 160° C. in air) lint, placed on this surface, and a sterilised watch glass strapped over it with adhesive plaster. After critical sweating, circle of lint removed, placed between two sterilised watch glasses held in a metal frame, and sent to me at laboratory. There each circle of lint placed in a separate broth-tube numbered, dated, and incubated at 37° C. After five days' incubation, agar slopes inoculated zig-zag from each, were incubated at 37° C., and examined daily for growth; if sterile, original broth-tubes were inoculated with *M. melitensis*, returned to incubator for four days, and then fresh slopes inoculated from them; on these *M. melitensis* invariably appeared, thus proving that sufficient disinfectant to prevent growth of *M. melitensis* had not been carried into circles of lint from disinfection of skin surface.

Nineteen sweat swabs from different patients were thus examined. In some cases the tubes remained sterile, in others the agar slopes yielded growth in discrete colonies.

Result.—No *M. melitensis* was ever recovered by this method.

Second Method.—The critical sweat was collected in sterile pipettes from four different patients, zig-zagged on agar and incubated. The collection was done by the sisters in the ward, who were supplied with the pipettes ready for use, and instructed how to break the point and apply them. They stated it was rare for sweat to collect in such large drops as to admit of collection in this manner, hence specimens were obtained from only four patients.

Result.—No *M. melitensis* was obtained.

Third Method (a modification of the first).—Circles of lint were obtained saturated with critical sweat from Malta Fever patients as in first method, but instead of being incubated in broth-tubes, were placed each in a 5 c.c. sterile normal salt solution tube, in which they were thoroughly agitated and ground up with a sterile glass rod, and the resulting fluid plated out in agar Petri dishes both by spreading ½ c.c. of it over whole surface, and by describing a centripetal spiral with a loopful of the fluid. Discrete colonies were always thus obtained after incubation at 37° C. The critical sweat of seven patients have been thus examined without *M. melitensis* having been obtained.

Fourth Method.—The circles of lint saturated with sweat obtained as in the first method were each placed in a separate broth-tube which was incubated at 37° C. for seven days; then a loopful was taken and placed on the surface of nutrose-glucose-litmus-agar in a Petri dish and spread over it by means of a Klein's platinum spreader, which, after completely going over the agar surface, was straightway passed over the surface of a second similar Petri plate. These plates were then incubated for five days, after which they were examined carefully for possible *M. melitensis* colonies. A total of 81 specimens were thus examined.

Result.—No *M. melitensis* were recovered.

Fifth Method (a modification of the third).—It seemed not unlikely that, supposing *M. melitensis* to be present in the circles of lint saturated with critical sweat, that it would be more likely to be obtained directly without previous incubation if nutrient broth were used instead of the salt solution, specified in Method 3; so, accordingly, the circles of lint were placed in separate broth-tubes, and thoroughly stirred up and agitated therein with a sterile Klein's spreader, followed by a vigorous shaking. Then one platinum loopful was immediately spread over an agar plate and incubated for five days at 37°, and then examined for possible *M. melitensis* colonies. The broth-tubes containing the sweat-saturated lint were incubated seven days, and then one loopful spread over two agar plates. These similarly incubated five days and then examined.

A total of 24 specimens were thus treated, but no *M. melitensis* was ever obtained on either series of plates.

Sixth Method.—At this stage of the examination the results of other experiments (see Section on Vitality of *M. melitensis* outside the Body) indicated that *M. melitensis* could not be expected to be recovered after three days in a nutrient medium containing other organisms; so consequently the procedure of the fifth method was modified by reducing the period of incubation of the sweat-saturated lint in broth to three days instead of seven, being otherwise identical.

A total of 30 specimens were so examined, but again no *M. melitensis* was ever obtained from either the plates inoculated the day the specimen was received, or from those inoculated from the broth-tubes after three days' incubation.

Seventh Method.—Instead of grinding up and shaking the sweat-saturated piece of lint in nutrient broth, and immediately plating a loopful of this, the piece of lint as received was placed flat on the agar surface of a Petri dish and pressed well on to the agar with a sterilised Klein's spreader, then it was lifted up with a pair of sterilised forceps and removed with the same surface downwards to the next adjacent area of agar, and there again pressed on to the agar; this process was repeated until the whole of the agar surface of the Petri dish was covered with "impressions" made from one surface of the piece of lint, usually 30 to 40 for a 10-centimetre Petri. Now another agar Petri was taken, and the same process repeated, but with the other surface of the lint, and both Petri dishes put in incubator at 37° C.; this completed, the piece of lint was then put in a broth-tube and incubated three days at 37° C., and then one loopful plated over two agar Petris.

A total of 86 specimens were thus examined, but no *M. melitensis* was ever obtained either from the direct series of "impression" plates or from the broth-tubes containing the circles of sweat-saturated lint. Frequently in the course of the examination one met with the colonies of *plus* Gram-staining glucose fermenting staphylococci, which turned up almost invariably in these bacteriological examinations of the skin.

The accompanying table (pp. 41, 42) shows the patients and the day of disease on which a specimen was taken; it will be seen that practically every day of the disease is represented by one or more examinations. The numbers on the top line indicate the day of disease, one column being given to each; the sign × indicates that an examination was made, being placed in the vertical column of the day of disease, and in the horizontal column appropriated to the name of the patient from whom it was taken.

It was found practically impossible to obtain sweat in such quantity as to admit of satisfactory injections into animals, but the number of specimens (251) examined, covering every period of the disease, and the varying methods employed, some of which succeeded so admirably in the isolation of *M. melitensis* from the blood and the urine of patients, practically justify the assumption that *M. melitensis* is not excreted in sweat of Malta Fever patients, or it would have been recovered in one of these numerous attempts.

Result.—*M. melitensis* has not been recovered from the 251 specimens of sweat examined, and in all probability is not excreted in this secretion.

Table showing Cases and Day of Disease on which Sweat was Bacteriologically Examined.

Patient's name.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Pigott.........																																								×							
Wilson									×																																						
Hagger																								×															×								
Kelly													×																																		
Markham ...									×								×																														
Mayes																			×								×																	×			
Francis																								×								×															
Lawrence ...																																				×											
Martin																																														×	
Jones																																															
Hewett																					×																										
Pudney																																													×		
Curry								×																											×	×	×	×	×	×		×					
Vincent																																															
Rivers																														×																	
Hurrell																																													×		
Anthony ...								×	×																							×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Marchant ...						×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	×		×
Silburn											×	×	×	×	×	×	×	×	×	×	×	×	×	×																							
Kinsella......																																	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Turner																																															
Anderson ...																																															
Dennis																																										×	×	×	×	×	×
Derby.........			×	×	×	×	×	×	×	×	×	×	×	×	×	×	×																														
Ericson																							×	×	×	×	×																				

Table showing Cases and Day of Disease on which Sweat was Bacteriologically Examined—*continued.*

Patient's name.	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102
Pigott.........																																														
Wilson	×																																													
Hagger																																														
Kelly																																														
Markham ...																																														
Mayes																																														
Francis																																														
Lawrence ...																																														
Martin	×																																													
Jones								×																																						
Hewett																																														
Pudney																																														
Curry																																														
Vincent																								×																						
Rivers																																														
Hurrell																																														
Anthony ...	×	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×					×		×	×													
Marchant ...	×	×	×				×		×		×		×	×	×	×		×																												
Silburn																																														
Kinsella ...	×	×	×	×	×	×	×	×	×	×		×	×	×																																
Turner										×	×	×																							×	×	×	×	×	×						
Anderson ...										×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×									
Dennis	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×																														
Derby.........																																														
Ericson																																														

III. ON THE VITALITY OF THE *MICROCOCCUS MELITENSIS* OUTSIDE THE BODY IN DIFFERENT ENVIRONMENTS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission, Malta.

In attempting to ascertain the presence or otherwise of *M melitensis* in the skin, sweat, breath, etc., of patients suffering from Malta Fever, it was obviously of some importance, having regard to the slow growth of this organism, as compared with the rapid growth of others in nutrient broth, a medium which could not be dispensed with, to ascertain for how long it could be recovered when incubated in broth in association with other micro-organisms, and accordingly the following experiments were undertaken, a control inoculation of the *M. melitensis* used being made into a tube of the same broth to verify its viability, the same generation of *M. melitensis* being used throughout.

A. *Vitality of* M. melitensis *in Mixed Broth Culture.*

No. 1. A sterile broth tube was inoculated with *M. melitensis*, and also from a broth tube (similarly with the same platinum loop) which had been allowed to become contaminated by exposure in the laboratory. This tube was well shaken and then one loopful was distributed over the surface of three successive agar Petri dishes with a Klein's spreader, and then tube and plates placed in incubator at 37°; each day another series of three plates was similarly inoculated from the broth tube, which was each time returned to the incubator, and after a period of five days' incubation, each set of three Petris was carefully examined for *M. melitensis*. This was found in the plates inoculated from the mixed broth culture after it had been incubated seven days, but not later.

No. 2. A repetition of No. 1. The result was the same. *M. melitensis* was recovered from the mixed culture for seven days, but no longer, in both cases the mixed culture at the termination of the experiment, 15th day of incubation, was slightly alkaline to litmus.

Nos. 3 and 4. On the same lines as No. 1; but in these two the mixed culture was composed of *M. melitensis* plus organisms derived from sweat, skin, and urine of Malta Fever cases, as far as possible equal quantities of each being taken. In No. 3, *M. melitensis* was recovered after two days' incubation, not later. In No. 4, started a week later, but with skin, sweat, and urine organisms from different sources, *M. melitensis* was not recovered at all. In both the reaction of the mixed broth culture was acid to litmus at the termination of the incubation (seven days).

Nos. 5, 6, 7, 8, 9, and 10. In each of these, performed successively, not collectively, the same procedure as in No. 1 was followed, the organisms used being *M. melitensis*, and cultures derived from skin, breath, and sweat. In none was *M. melitensis* recovered after more than *one* day's incubation, and in all the mixed broth culture was acid to litmus at the end of each incubation.

Result.—M. melitensis incubated in broth in presence of other organisms is recoverable for a very short time, seven days, in presence of alkali producing organisms. One to two days in presence of acid-producing organisms contrasting greatly with its recoverability in pure broth culture, from a tube of which, inoculated December 12, 1904, it was recovered by a sub-culture on agar, April 25, 1905, an interval of over four months.

B. *Vitality of* M. melitensis *in Pure Culture.*

1. *On agar agar dry.*—Two agar slopes inoculated with *M. melitensis* March 29, 1904, which had been incubated at 37° C. for four days and then placed aside in laboratory cupboard with cotton wool plug unprotected by a rubber cap, had on December 30, 1904, become so dry that no colony could be detached for sub-culture, and the agar itself had contracted to a thin shred; sterile broth was therefore added to the two tubes until the upper level of the dry culture was submerged, these were then placed in the incubator at 37° C. till January 4, when the broth of one had become turbid, this was now sub-cultured, and pure *M. melitensis* was recovered and verified. No growth was obtained from the other.

Two similar slopes inoculated April 5, 1904, examined January 24, 1905, failed to give any growth. Two other such slopes inoculated April 21 and 24, 1904, similarly examined March 20, 1905, failed to give any growth.

Result.—M. melitensis had remained alive and capable of reproduction in a dried-up condition on agar from March 29 to December 30 = 276 days (nine months).

2. *In Litmus Milk.*—A tube of litmus milk inoculated with *M. melitensis* (Second Generation, Human Spleen, Bowles), December 12, 1904, yielded *M. melitensis* in sub-culture April 26, 1905, a period of over four months, but in very small quantity, a loopful which in January and February had yielded colonies by the hundred, now giving only 1 to 10 colonies; and after May 5 ceased yielding colonies though experimented with for a fortnight longer, hence, presumably dead after 144 days of vitality.

3. *In Nutrient Ordinary Beef-Peptone Broth.*—A tube of this was inoculated from same source at same time as litmus milk, December 12,

1904, and right up to June 3, 1905, each loopful taken twice weekly for sub-culture was yielding a plentiful supply of *M. melitensis.*

Result.—Still alive and actively reproductive after $5\frac{2}{3}$ months in nutrient broth.

In all these the media were titrated to a reaction of + 10 of acidity with phenol-phthaleine (Eyre's scale).

Results.

M. melitensis	lived on dry agar	....	276	days.
,,	,, in litmus milk	....	144	,,
,,	,, nutrient broth	....	173	,,

C. *Vitality of* M. melitensis *in Urine.*

A noteworthy feature of the urine of Mediterranean Fever patients is the length of time it remains acid after it has been passed; the following observations given in tabular form demonstrate this. These urines were taken from patients in the wards without special precautions and were kept in the laboratory cupboard, again without any special precautions. The acidity was determined each time by titration against a standard $\frac{N}{5}$ solution of potassium hydrate, phenol-phthallein being used as the indicator, and is expressed according to Eyre's scale. It will be remembered that the optimum reaction of culture media for *M. melitensis* has been found to be an acidity of +10, Eyre's scale (see Part I of these reports).

Patient's name.	Date urine passed.	Acidity when passed.	Acidity on Jan. 30.	Acidity on Feb. 21.	Acidity on Mar. 29.
Anderson	Jan. 3	+36		+36	+26
Turner...............	3	+ 5		+ 2	− 2 alkaline
Martin...............	3	+44	+44	+40	+18
Rentcombe (*a*) ...	3	+60		+28	+ 4
,, ...	12	+30		+12	+12
,, (*b*) ...	15	+52	+40		
Webb (*c*)............	Feb. 5	+60			+50
Jacombe (*d*)	5	+32			+32

In the following observations on the life of *M. melitensis* in various specimens of urine (again given in tabular form), the following was the method adopted. The specimens of urine were some healthy and some obtained from Malta Fever patients, and one of each sterilised in autoclave at 115°. In each case, after the acidity of the specimen had been determined, 10 c.c. of it were placed in a sterile test-tube with the usual wool plug. As it was also considered of importance to have information as to the number of colonies of other micro-organisms these specimens contained, each was well shaken and one loopful, taken with a standard loop, distributed over the surface of an agar Petri dish, which was then incubated at 37°, and the number of colonies counted and recorded five days later. After the abstraction of this one loopful, each specimen was inoculated with *M. melitensis*. The same brand was placed in each, a four days' growth on glucose litmus agar of the second generation of *M. melitensis* obtained from the spleen of a fatal case (Bowles) and, as far as possible, the same amount of culture in each case, a small platinum loop being set aside for the purpose of delimiting each time the area of agar to be denuded of growth. The tubes of *M. melitensis* inoculated urine were now placed in the laboratory cupboard (temperature about 15° C.) and daily well shaken and a loopful from each plated on agar, the plate incubated for five days and then examined for *M. melitensis*, which, if found, was verified in the usual way. It was found that after a variable number of days, some morning, a plate from a given urine would contain no *M. melitensis*: this was regarded not as a sign of death of all the *M. melitensis* in the specimen, but as indicating a great diminution in number, and the daily plating persevered with till there had been a succession of seven blank days. Not infrequently a specimen would yield *M. melitensis* one day, then not yield it for one or two days and then again give it. After a succession of seven days' plating without recovery of *M. melitensis*, that particular observation was terminated with the then acidity of that particular specimen being determined and recorded.

The following were the results obtained :—

Source of urine used.	Reaction.	No. of colonies per loop.	No. of days *M. melitensis* was recovered.	Reaction of urine at end of observation.
Unsterilised, normal healthy ...	+ 7 acid	57	2	Very alkaline.
" " " ...	+ 8 "	35	5	Just neutral.
" " " ...	+ 7 "	57	2	Alkaline, strongly.
" " " ...	+ 8 "	5	33	− 5 alkaline.
Unsterilised, Malta Fever patient	+18 "	13	15	−40 "
(*a*) " " "	+60 "	32	24	−25 "
(*b*) " " "	+52 "	7	18	−25 "
" " "	+40 "	6	43	Just neutral.
(*c*) " " "	+60 "	175	36	+16 acid.
(*d*) " " "	+32 "	51	49	+ 4 "
Sterilised, normal healthy.........	+ 8 "	Nil	17	+ 2 "
Sterilised, Malta Fever patient...	+40 "	"	33	+20 "

A control inoculation of the same culture of *M. melitensis* into nutrient broth, kept under same conditions, was recovered by sub-culture on to agar after 4½ months.

Remarks.—Here neither differences in acidity or in number of other organisms seem to have had an appreciable influence on the duration of life of *M. melitensis* in urine, variations in this being presumably due to variations in other constituents so far as urine derived from Malta Fever patients is concerned, though there does seem to exist a direct connection between duration of life of *M. melitensis* and number of other organisms in the case of normal healthy urine, the greater the number of the latter the shorter the life of *M. melitensis* in such urine containing both. The urines lettered (*a*), (*b*), (*c*) and (*d*) in the two Tables I and II were identical, and the date of last determination of acidity in Table I was after the final one in Table II. The factor of difference was the presence of *M. melitensis* in urines of Table II and its absence in those of Table I, and I think the development of alkalinity in the urines of Table II compared with its non-development in the identical urines of Table I is attributable to the presence of *M. melitensis* in those of Table II, this being in accord with other observations on production of alkalinity by *M. melitensis* in nutrient media (*see* Part I of these reports).

The salient feature, however, is the comparatively long retention of reproductive activity of *M. melitensis*, lasting as long as seven weeks, in the urine of Mediterranean Fever cases. I may remark here that I find that 23 out of 30 samples of urine from Mediterranean Fever cases examined effected agglutination of *M. melitensis* in varying degrees; evidently agglutinins are excreted in the urine.

D. *Vitality of* M. melitensis *in Diluted Urine.*

1. The same brand and quantity of *M. melitensis* was placed in 1 c.c. of *fresh healthy urine*, which was well shaken and then added to 100 c.c. of sterilised tap water contained in a flask with cotton wool plug, the idea being to simulate the diluted fluid of the ordinary urinal minus accessory contaminations. Here again the flask was daily well shaken and a loopful plated on agar, the plate incubated and examined for *M. melitensis* colonies in the usual way. *M. melitensis* was recovered for nine days.

2. The same experiment was repeated, using *urine* from a *Malta Fever case*, and *M. melitensis* was recovered day by day, with occasional intervals of one, two, or three days, for 79 days; the daily sub-culture was persevered with for 14 days longer without *M. melitensis* being recovered.

Result.—1. *M. melitensis* was recovered from diluted healthy urine for nine days.

2. *M. melitensis* was recovered from diluted Mediterranean Fever urine for 79 days.

E. *Vitality of* M. melitensis *in Urine—Contaminated Milk.*

1. The same brand and quantity of *M. melitensis* was placed in 1 c.c. fresh healthy urine, which was well shaken and then added to 100 c.c. of sterilised goat's milk contained in a wool-stoppered flask, which was thoroughly well shaken every morning, and then a loopful plated on agar, incubated and examined for *M. melitensis* colonies. *M. melitensis* was recovered for three days, but after that was completely crowded out by other colonies.

2. The same experiment was repeated, using *Malta Fever urine* instead of healthy urine. In this case *M. melitensis* was recovered for 38 days.

Result.—1. *M. melitensis* recovered from milk contaminated with healthy urine for three days.

2. *M. melitensis* recovered from milk contaminated with Mediterranean Fever urine for 38 days.

F. *Vitality of* M. melitensis *in Urine Dried on Fabrics.*

The intention was here to obtain information as to the possible infectivity of garments soiled with urine containing *M. melitensis.*

1. Ten c.c. of normal healthy urine were taken and inoculated with the same brand and quantity of *M. melitensis* as in the preceding urine experiments, pieces of sterile lint were immersed in it till saturated, then removed and allowed to dry in a sterile Petri dish at the laboratory temperature (about 15° C.), which took four days. Then daily two small pieces were snipped off with sterile scissors, one put in a 10 c.c. broth tube, the other used to make impressions on the surface of agar in a Petri dish, by lifting it from area to area of the agar with a pair of forceps, and in each new situation pressing it on to the surface of the agar with a platinum spreader. The broth-tube and plate were then incubated and examined for *M. melitensis* in the usual way, but in this experiment none were recovered.

2. Precisely the same experiment, but using navy blue serge No. 3, such as is worn by the bluejacket. Again the result was the same, no *M. melitensis* was recovered.

3. Thinking that the failure to recover *M. melitensis* in the two preceding experiments might be due to the very slow drying on the fabric and the consequent facility for fermentation of the urine which certainly took place, the same experiments were repeated, using Malta Fever urine and drying the fabrics in the incubator at 37° C.; this was found to take only 24 hours instead of the four days requisite at atmospheric temperature.

Result.—*M. melitensis* was recovered from the lint so treated for five days, and from the blue navy serge for 78 days; daily sub-inoculations having been made as described in F 1. The difference between the periods of recovery in the two cases may doubtless be attributable to the very different modes of manufacture of the two fabrics.

G. *Vitality of* M. melitensis *in Sterilised Tap Water.*

Ten c.c. of ordinary tap water were taken in a test-tube with cotton wool plug and sterilised in autoclave at 115° and then inoculated with same brand and quantity of *M. melitensis* as in the urine experiments, C 2, and placed in laboratory cupboard at temperature of about 15° C. Each day this tube was well shaken and cultured as described in C, by means of standard platinum loop and spreader on agar in Petri dishes.

Result.—*M. melitensis* was recovered for 50 days.

H. *Vitality of* M. melitensis *in Unsterile Tap Water.*

The same experiment as G, but the tap water not sterilised and two observations were made.

1. Commenced December 12, finished December 29. *M. melitensis* recovered for 10 days, tap water from rain tank on roof being used; this is not usually considered potable.

2. Commenced December 30, finished March 23. *M. melitensis* was recovered for 72 days, tap water from ordinary urban house-supply being used, which is used for drinking purposes.

Result.—1. *M. melitensis* was recovered from tank water for 10 days.

2. *M. melitensis* was recovered from potable water for 72 days.

I. *Vitality of* M. melitensis *in Unsterile Sea Water.*

This experiment was made twice in the same way as those described under G and H, the same brand and quantity of *M. melitensis* being used. The sea water was obtained from the area of the Grand Harbour in which H.M.S. "Egmont" (a stationary depôt ship on board of which a varying number of 300 to 600 men are living) is moored, and in close proximity to this ship, with the sewage of which it was demonstrably fouled.

Result.—1. The first specimen of sea water yielded three colonies per loop of other micro-organisms before inoculation, and *M. melitensis* was recovered from it after inoculation for 46 days.

2. The second specimen of sea water yielded 11 colonies per loop of other micro-organisms before inoculation, and *M. melitensis* was recovered from it after inoculation for 11 days.

J. *Vitality of* M. melitensis *Dry on Cover Slips.*

A large number of cover slips were cleaned and sterilised, an emulsion of same brand of *M. melitensis* as that used in all the preceding experiments made in sterilised distilled water, and one drop of this placed with standard platinum loop on one surface of each cover slip, these being arranged in rows inside sterile Petri dishes wherein the drops of emulsion were allowed to dry, the whole being kept in laboratory cupboard at about 15° C. Each day one was removed with a pair of sterile forceps and placed, *M. melitensis* film-side downwards, on the surface of agar contained in a Petri dish, over which it was moved by the forceps till all the *M. melitensis* film had apparently been left distributed over the agar surface, when it was left *in situ*, still with its "film" side adhering to the agar, the cover of the Petri replaced numbered and dated, and the whole incubated for five days and then examined for growth.

Result.—M. melitensis was recovered in this way from these cover slips for 15 days; a result of some importance as showing the inherent vitality of *M. melitensis* even when dried and separated from any trace of organic matter. It will be remembered that in the dry condition on organic matter (nutrient agar) it lived for over nine months (*vide* Section B of these experiments).

K. *Vitality of* M. melitensis *in Earth.*

1. *In Sand Free from Organic Matter.*—The sand used was a silicious red sand obtained from North Africa; it was heated to redness to burn off organic matter, then well shaken with distilled water, the reaction of which was after this found to be neutral. Some of it was then sterilised in dry air at 160° C. inside a pair of watch glasses held in a clip. An emulsion of the same quantity and brand of *M. melitensis* as in preceding experiments (Second Generation from Human Spleen, Bowles) made in 5 c.c. of distilled sterilised water and well mixed with the sterilised sand, and the whole allowed to dry in a cupboard at the temperature of the laboratory, 15° C. Twice a week two specimens were put out to incubate as follows: (*a*) A little of the inoculated sand was put in a 10 c.c. broth tube which was then incubated for five days, after which a loopful was put out on a glucose-litmus-agar slope which was incubated and examined for growth; (*b*) A little was put on the surface of similar agar in a Petri dish, sufficient nutrient broth added to make a mud of it, and this was then spread out with a Klein's platinum spreader, and, after five days' incubation at 37° C., examined for growth. I found that this method gave just as constant results as the former (*a*), and it had the advantage of saving one incubation and the corresponding number of days in time.

Result.—M. melitensis was recovered in this manner for 16 days.

Remark.—This experiment and the last are quite comparable in that the *M. melitensis* culture used was not only the same, but that it was kept dry with an inorganic environment free from organic matter, and the duration of reproductive vitality was much the same; on cover slips 15 days, in sand 16 days.

2. *In Various Malta Soils.*—In preliminary experimentation on the sterilisation of these, they were all found after sterilisation by dry heat to be excessively alkaline, a condition seriously prejudicial to the vitality of *M. melitensis.* This was found to be due to the large amount of calcium carbonate they contained, some of which, by the dry heat used, was converted into calcium oxide, which on the addition of water became calcium hydrate. This caused one to examine the reaction of the various soils as received, by thoroughly stirring up and shaking each, and then shaking a little in distilled water in a test-tube and taking the reaction of that. All the specimens were thus found to be slightly alkaline to begin with. So sterilisation was now done by putting the specimen of soil in a beaker, half filling this with distilled water and placing it in the autoclave at 115° for 30 minutes; this was found to effect sterilisation without any alteration of alkalinity.

(a) *In Greyish Yellow Soil, with additional Organic Matter.*

This soil was personally obtained from a field in Sliema, was sterilised as just described, sterility verified by broth culture, a portion in bulk equal to about 10 c.c. placed in a sterile test-tube and put in incubator at 37° C. till dry, then on to it was poured, by means of a sterile pipette, a broth growth of *M. melitensis* (still Second Generation from Human Spleen, Bowles), till its upper $\frac{1}{3}$ was quite saturated with moisture, then tube was plugged with sterile wool and placed in laboratory cupboard. Twice in each week two portions from the upper surface were planted out in the manner described in K 1 for sand, incubated, and examined for growth. It was noticed, as the experiment progressed, that the upper surface of the soil got apparently dry, but that at the bottom of the test-tube it became damp from percolation of the nutrient broth downwards, and this dampness persisted till the conclusion of the experiment; so that the *M. melitensis* present must have been continually in the presence of water vapour. As in K 1, it was found that planting out on agar, as described, gave just as constant results as planting out in broth and then sub-culturing from this.

Result.—*M. melitensis* was thus recovered from this soil for 91 days.

(b) *In Reddish Soil without additional Organic Matter.*

This soil was also personally obtained from a Sliema field; it was very similar in composition to that used in the preceding experiment, differing mainly by containing a little under 1 per cent. of iron oxide, to which its reddish colour was due. It was sterilised under water in the autoclave at 115° C., sterility verified by broth culture, and sufficient placed in a small sterile Petri dish (3 cm. in diameter) to give a depth of about $\frac{3}{16}$ to $\frac{1}{4}$

of an inch. An emulsion of same quantity and brand of *M. melitensis* as in Experiment K 1 was made in 5 c.c. of distilled sterilised water, and, by means of a sterile pipette, was distributed all over the surface of the sterilised soil contained in the Petri dish. Two small portions of this were planted out in agar and in broth twice weekly in the manner already described, incubated and examined for growth.

Result.—M. melitensis was thus recovered from this soil for 80 days.

(c) *In White Soil without additional Organic Matter.*

This specimen of soil was obtained from an area of land where building operations were going on, and consisted very largely of the *débris* from the stone cutting, shaping, and smoothing operations which, as is usual in Malta, were carried out on the spot where the finished stone was wanted for use. It is a very soft friable Globigerina limestone, and the *débris* used contained only a mere trace of organic matter. With this the experiment just described was exactly repeated in every particular.

Result.—M. melitensis was recovered for 24 days.

(d) *In Recently well-manured Soil, Sterilised and kept Wet.*

This experimênt was intended to contrast with the latter in the amount of organic matter present in the soil, it being very great in this experiment, very little indeed in the last one, and also in the amount of water present, a similar difference being maintained. Recently (five weeks) manured soil was obtained from the Argotti Botanical Gardens, dried, pulverised in a mortar, sterilised as in the preceding three experiments, an amount equal in bulk to about 10 c.c. put in a sterile test-tube and well shaken down; and then on to it was poured, by means of a sterile pipette, an emulsion of *M. melitensis* grown on agar made from the same quantity, brand, and generation of *M. melitensis* as in the preceding experiments, in 5 c.c. of distilled sterilised water. This soil was further kept saturated with moisture by dropping on it from time to time sterilised tap water from a sterile pipette, and was kept at laboratory temperature (15° C.) for the whole period of the experiment. From it two portions were planted out in broth, and on agar twice weekly in the way described in K 1, then incubated and examined for growth. The experiment was started on December 14, 1904, and each planting out yielded *M. melitensis*, which, as usual, was duly verified. During the examination and verification of the growth of January 8, it was noticed that the resulting colonies, while resembling the usual *M. melitensis* colony in every other particular, had less sharply defined, less abrupt margins, and that the microscopical preparations contained a few bacillary forms. By February 12 the new colonies of that date presented slightly crenated edges, shading away on the agar, though quite similar in size and shape to standard *M. melitensis* colonies of same duration of growth on same agar, and now consisted almost entirely under the microscope of small bacillary forms, when stained, of about $\frac{2}{3}$ the diameter of a normal *M. melitensis*, and three times its length. A sub-culture of this was now put through *all* the tests specified in Part I of these Reports, for the recognition and verification of *M. melitensis*, behaving in all particulars like standard *M. melitensis* save in the morphological details mentioned. These bacilli in hanging-drop preparations were feebly motile. Many specimens were stained for flagellæ according to Rossi's method, but none were demonstrated. It will be remembered that Gordon, in a paper in the *Lancet*, March 11, 1899, described flagellæ in connection with *M. melitensis.* I have not succeeded in verifying this. Successive sub-cultures of this growth of February 12, for 10 generations, in broth and on agar were now made, but there was no reversion to the coccal form; the Tenth Generation was exactly like the First; but a sub-culture from the Ninth Generation into peptone water (made for the purpose of ascertaining the presence or absence of indol and nitrite formation, neither present), showed in a stained specimen both cocci and bacilli, and intermediary forms such as French bacteriologists speak of as "cocco-bacille." On February 22 a rabbit which had never been experimented upon, and whose blood gave no trace of agglutination with standard *M. melitensis*, was injected intra-cerebrally with the usual aseptic precaution with $\frac{1}{2}$ c.c. of an emulsion made from this same Ninth Generation. Its temperature rose to 105° F. the same evening and 106° F. the following evening, after which it fell to normal and never rose again. On February 25 there was a distinct agglutination reaction on *M. melitensis*, with its blood serum in a dilution of $\frac{1}{32}$; this had increased on March 5 to a dilution of $\frac{1}{300}$. On February 28 1 c.c. of blood was taken aseptically from the animal's left internal saphenous vein, placed in 19 c.c. of nutrient broth, and incubated in the usual way. There being absolutely no trace of growth obtainable from this up to March 10, the animal, which had fully recovered from the operation, was that day chloroformed, a *post-mortem* made, and inoculations made into both broth and agar from the brain, heart's blood, urine, spleen, liver, and kidneys; no growth was obtained from any of these.

Concurrently with all this, the periodical plantings out had been carried on, but no growth was obtained from any planting out later than March 7, though these were continued till March 22.

Remarks.—A bacillary form in pure cultures of *M. melitensis* has been noticed by various workers, but not apparently in so marked a degree as in this instance. As an intra-cerebral injection of the coccal form of *M. melitensis* usually produces in a rabbit death in four or five days with presence of *M. melitensis* in the various organs, the bacillary form produced, as described, is obviously of less virulence.

Result.—M. melitensis was recovered from this sterilised, manured, and saturated soil rich in organic matter for 83 days, a bacillary form of *M. melitensis* deficient in virulence being developed.

(e) *In Recently Manured Non-Sterilised Soil.*

The same soil as in the last experiment was used and treated in precisely the same way save that it was not sterilised.

As it was anticipated that very rapid overgrowing of the *M. melitensis* put in (the viability of which was as usual tested by a control) would take place, a little was planted out daily, a small portion being placed on the surface of agar in a Petri dish, made into mud with nutrient broth, and distributed by means of a Klein's platinum spreader all over the surface of agar in three successive Petri plates. Although in this manner discrete isolated colonies were obtained in the third plate, no *M. melitensis* was ever recovered, though four separate repetitions of the experiment were made.

Result.—M. melitensis speedily crowded out by the other organisms present.

Summary of Results obtained as to Vitality of M. Melitensis *Outside the Body, the same Brand, Generation and Quantity of* M. melitensis *being used throughout* (*except in* B 1).

	Days.
A. In mixed broth culture with—	
1. Laboratory contamination	7
2. ,, ,,	7
3. Organisms derived from sweat, skin and urine	2
4. ,, ,, ,, ,,	Nil
5 to 10. ,, ,, ,, ,, and breath; in each	1
B. In pure culture media with a reaction of + 10 acid—	
1. On agar slope (source of *M. melitensis* not noted)	276
2. In litmus milk (source of *M. melitensis* as in all other experiments)	144
3. In peptone broth (ditto)	173
C. In urine (persistent acidity of Mediterranean Fever urine as described)—	
1. In unsterilised normal healthy urine, four experiments	2, 5, 2 and 33
2. ,, ,, Malta Fever urine, six experiments	15, 24, 18 43, 36 and 49
3. ,, sterilised normal healthy urine, one experiment	17
4. ,, ,, Malta Fever urine, one experiment	33

	Days.
D. In diluted urine—	
1. Healthy urine diluted 100 times with sterile tap water	9
2. Mediterranean Fever (ditto)	79
E. In urine contaminated milk—	
1. Goat's milk contaminated with 1 per cent. healthy urine	3
2. ,, ,, 1 ,, Mediterranean Fever urine	38
F. In urine dried on fabrics—	
1. In Mediterranean Fever urine dried on lint	5
2. ,, ,, ,, navy serge	78
G. In sterilised tap water	50
H. In unsterilised tap water—	
1. Tank water	10
2. Potable water	72
I. In unsterile sea water, two experiments	11 and 46
J. Dry on cover slips	15
K. In various earths—	
1. In sand free from organic matter	16
2. ,, various Malta soils—	
a. In sterilised grey-yellow soil with added organic matter	91
b. In sterilised reddish soil without added organic matter	80
c. In sterilised white soil almost free from organic matter	24
d. In sterilised well-manured soil rich in organic matter (bacillary forms of *M. melitensis* developed)	83
e. In non-sterilised well-manured soil	Not recovered

IV. ON THE RECOVERY OF *MICROCOCCUS MELITENSIS* FROM THE URINE OF MEDITERRANEAN FEVER PATIENTS.

By J. Crawford Kennedy, Captain R.A.M.C., Member Mediterranean Fever Commission, Malta, April, 1905.

Since September, 1904, this work has been more than trebled, and special attention has been paid to the quantities excreted and to the period of disease during which the excretion is greatest.

The following table is a summary of the work done :—

	No. of samples examined.	No. of times *M. melitensis* recovered.
September	347	6
October	217	6
November	581	63
December	398	43
January	201	19
February...............	110	19
March to April 2 ...	120	30
Total	1974	186

Percentage of recoveries...... 9½ per cent.

The number of cases examined was 61, and from 33 of these *M. melitensis* was recovered. Therefore *M. melitensis* was recovered from 54 per cent. of the cases examined. Deduct from this the cases which were examined less than 10 times—it leaves 50 cases and 31 recoveries, or 62 per cent.; 43 cases were examined over 20 times, with 31 recoveries, or 72 per cent.

The method of examination was the same as described by Major Horrocks in a former report, and each recovery put through the usual tests.

In order that the work may be taken in at a glance, I have prepared a list of the cases from which *M. melitensis* was recovered, giving particulars of number of samples and recoveries, quantity and period of disease, also a chart of the temperature. In those cases which supplied many recoveries the whole chart is given, in those with only one recovery, only the previous and subsequent two or three days' temperature is given.

List of Cases from which *M. melitensis* has been Recovered, giving particulars of Quantity and Period of Disease.

No.	Name.	No. of samples examined.	No. of times *M. melitensis* recovered.	Greatest No. of colonies *M. melitensis* found in a cubic centimetre urine.	No. of times *M. melitensis* recovered when temperature of previous or of subsequent 24 hours not above 99°.	Earliest and latest day of illness on which *M. melitensis* recovered.	Remarks.
1	Kinsella	97	27	189	16	21 111	See chart. This case examined all through illness. The excretion of *M. melitensis* was almost entirely during convalescence.
2	Bean	44	23	450	7	58 84	See chart. A normal temperature for 7 days during excretion of *M. melitensis*.
3	Ralph	76	18	440	2	39 133	See chart. Excretion of *M. melitensis* after 33 days' normal temperature.
4	Smith (Rifles)	64	6	309	—	74 114	See chart.
5	Gane	29	14	1068	7	108 145	See chart. Excretion after 27 days of practically normal temperature.
6	Charlton	35	4	18	4	82 102	See chart. Excretion after 30 days' normal temperature.
7	Bolt	151	23	129	—	74 165	See complete chart.
8	Anthony	91	3	Innumerable	—	81 156	See chart. Colonies so thick in one sample as to be uncountable—from 500 to 800 in one drop urine.
9	Surmin	93 (74 days)	48 (41 days)	Innumerable	27	174 249	See chart. Another case of a sudden gush of *M. melitensis* in urine.
10	Groom	70	1	3	1	82	See chart.
11	Mitchell	4	1	6	1	77	See chart. Excretion after 12 days' normal temperature.
12	Rivers	4	1	3	1	43	See chart.
13	Cannole	31	2	3	2	102 112	See chart. Excretion after 15 days' normal temperature.

List of Cases from which *M. melitensis* has been Recovered, giving particulars of Quantity and Period of Disease—*contd.*

No.	Name.	No. of samples examined.	No. of times *M. melitensis* recovered.	Greatest No. of colonies *M. melitensis* found in a cubic centimetre urine.	No. of times *M. melitensis* recovered when temperature of previous or of subsequent 24 hours not above 99°.	Earliest and latest day of illness on which *M. melitensis* recovered.	Remarks.
14	Campbell ……	71	2	6	—	93 94	See chart.
15	Walker ………	25	2	116	—	55 56	See chart.
16	Silburn ………	23	2	9	2	60 65	See chart. Excretion during convalescence.
17	Turner ………	80	1	3	—	52	See chart.
18	Bagwell………	29	1	3	—	77	See chart.
19	Renteome ……	40	1	4	1	99	See chart.
20	Marchant ……	55	1	15	—	72	See chart.
21	Donovan ……	46	1	3	1	57	See chart.
22	Bennett………	41	1	4	1	83	Excretion after 24 days' normal temperature; quite convalescent.
	Total ……	1199	183	Earliest day recovered ………		21	Out of 20 cases examined during convalescence 11 were found to be excreting *M. melitensis*.
	Average…	—	15·2 per cent.	Latest day recovered …………		249	

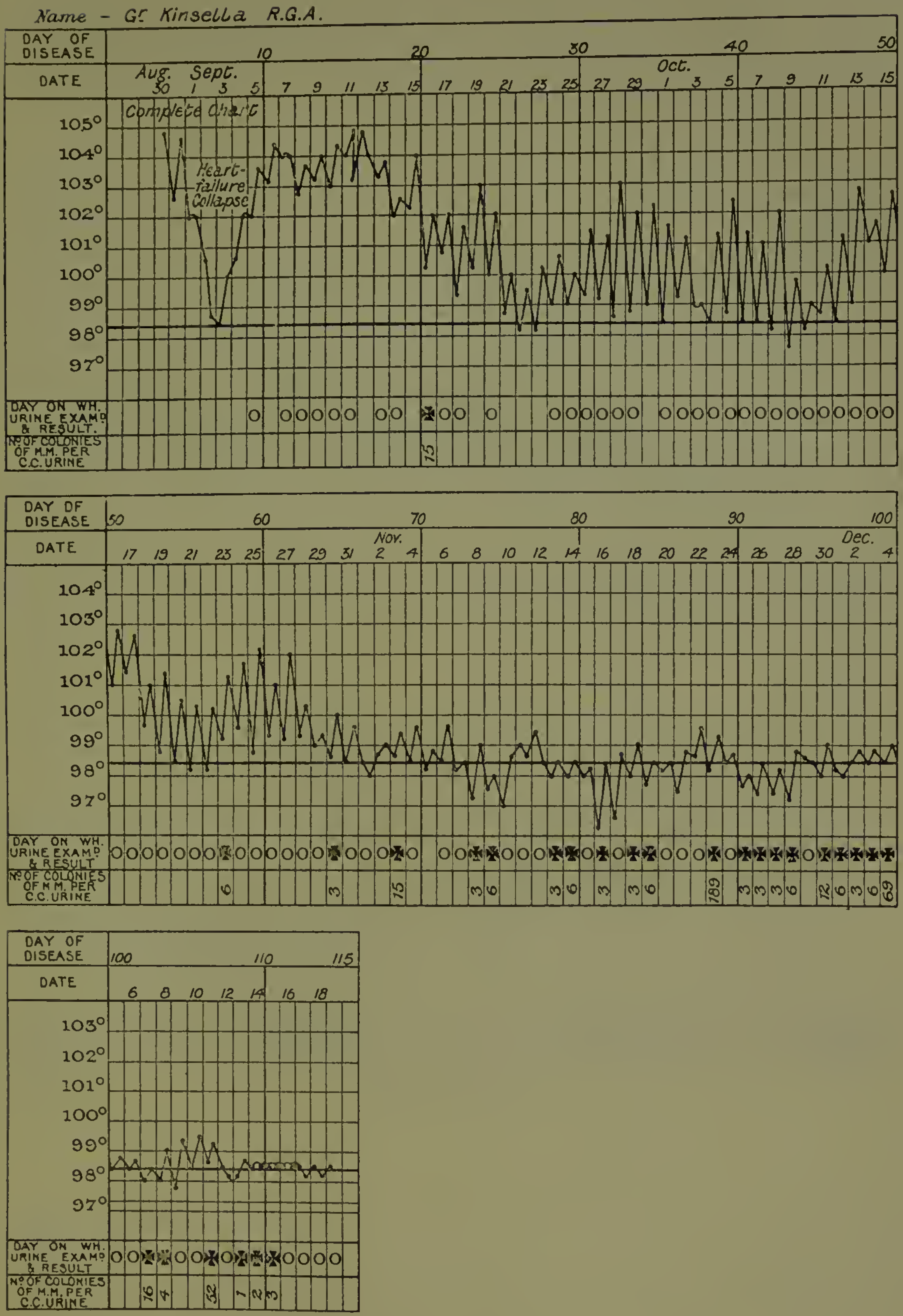


Chart 1.—KINSELLA.

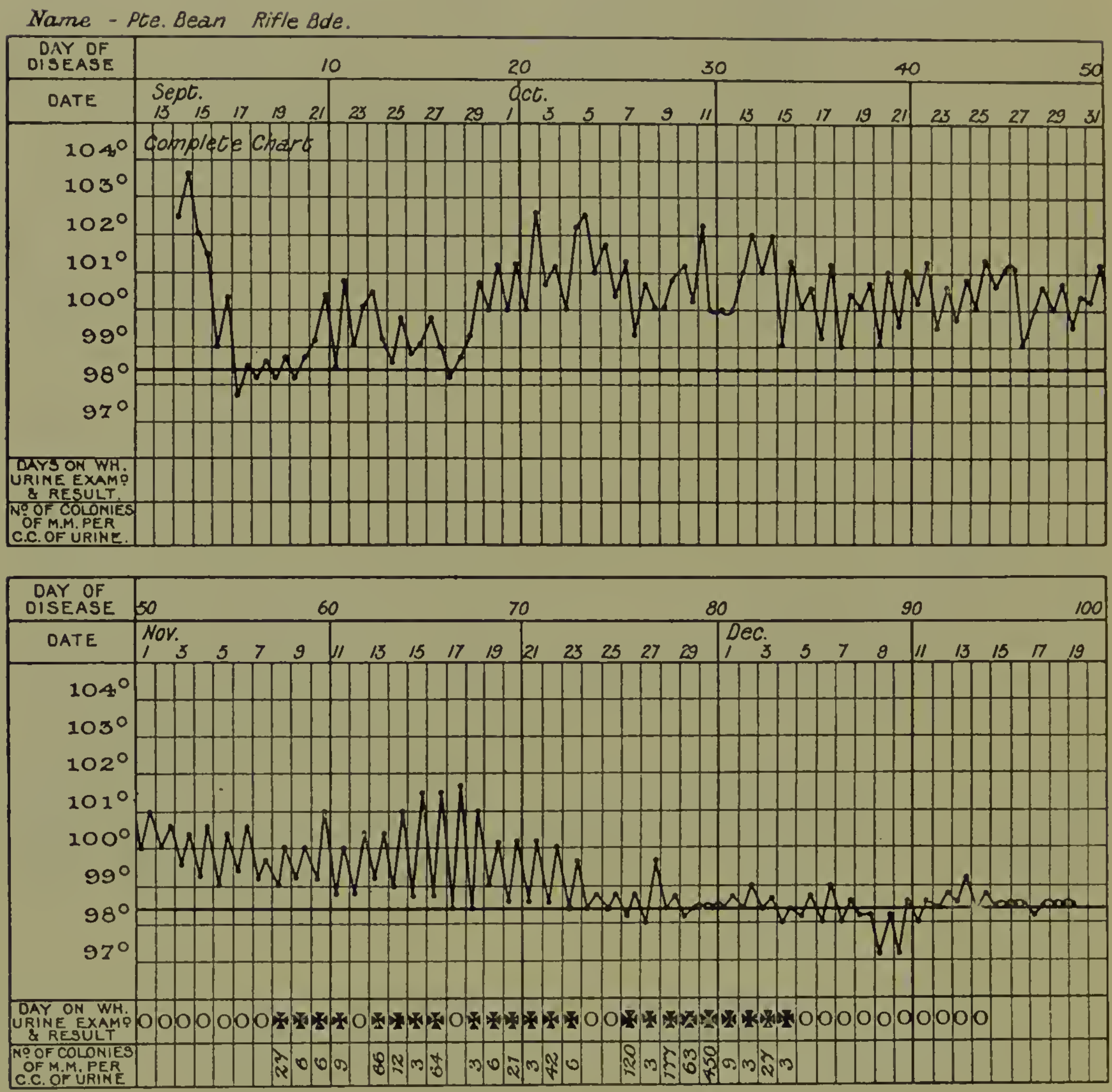

Name - Pte. Bean Rifle Bde.
DAY OF DISEASE
10
20
30
40
50
DATE
Sept.
13 15 17 19 21 23 25 27 29
Oct.
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
104°
103°
102°
101°
100°
99°
98°
97°
Complete Chart
DAYS ON WH. URINE EXAMº & RESULT.
Nº OF COLONIES OF M.M. PER C.C. OF URINE.
DAY OF DISEASE
50
60
70
80
90
100
DATE
Nov.
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Dec.
1 3 5 7 8 11 13 15 17 19
DAY ON WH. URINE EXAMº & RESULT
Nº OF COLONIES OF M.M. PER C.C. OF URINE
27 6 6 9 66 12 3 64 3 6 21 3 42 6 120 3 177 63 450 9 3 27 3

Chart 2.—Bean.

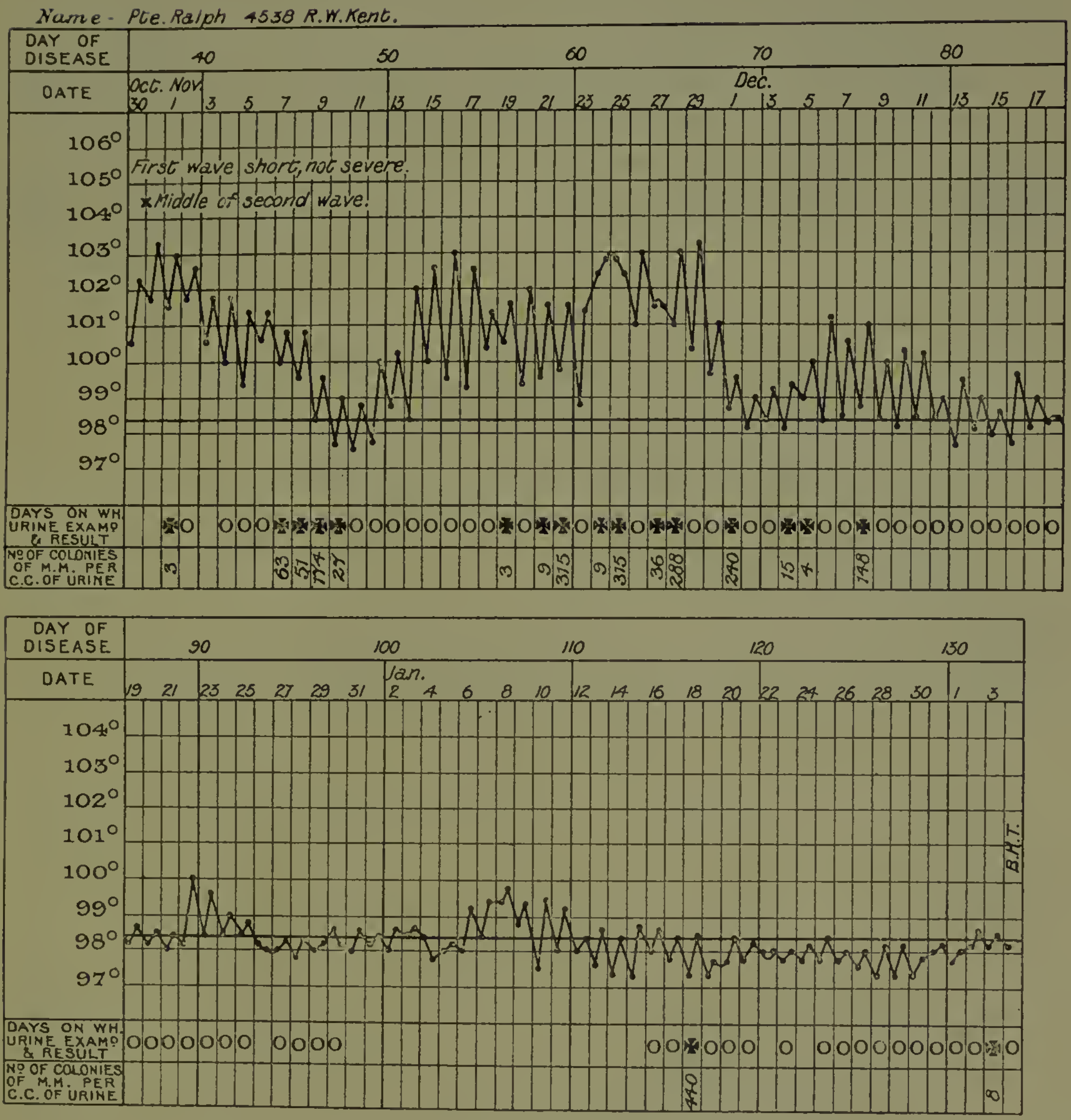


Chart 3.—Ralph.

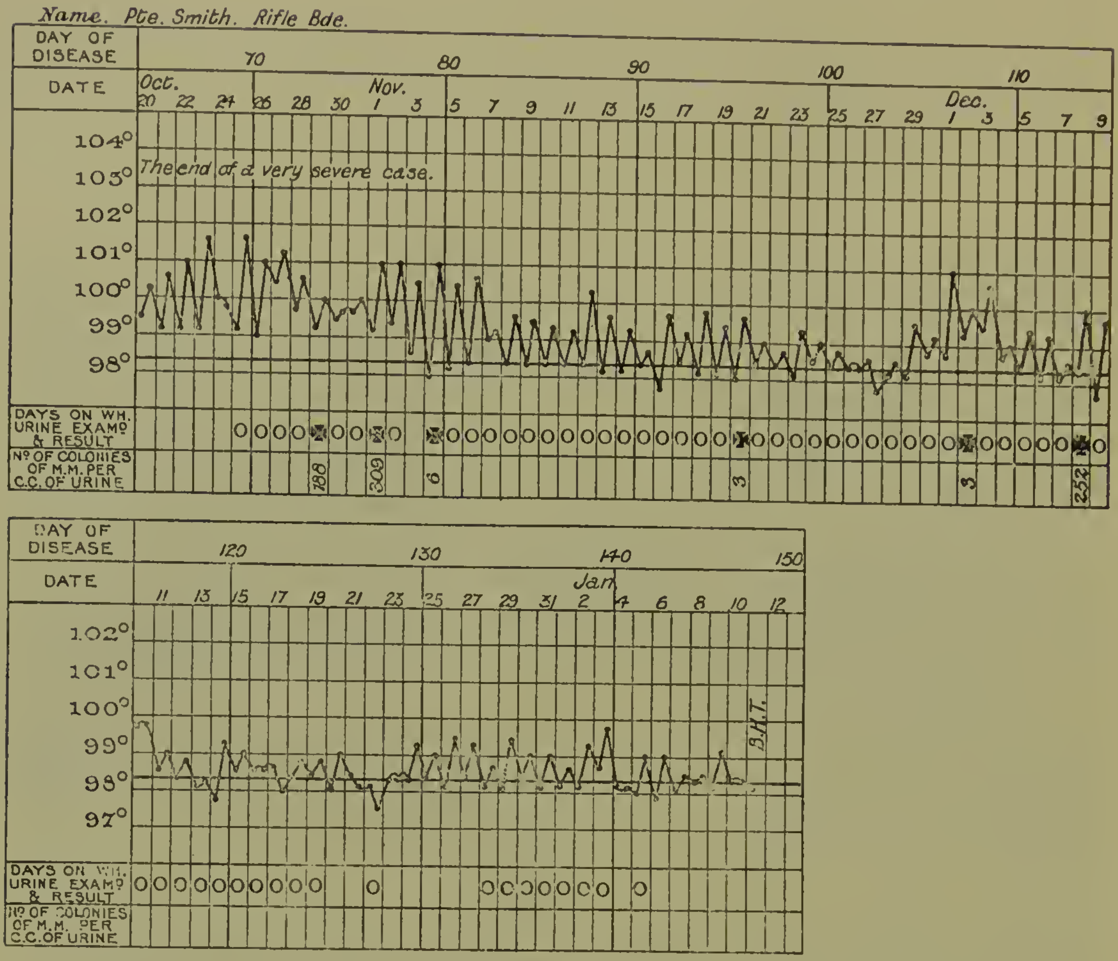


Chart 4.—Smith.

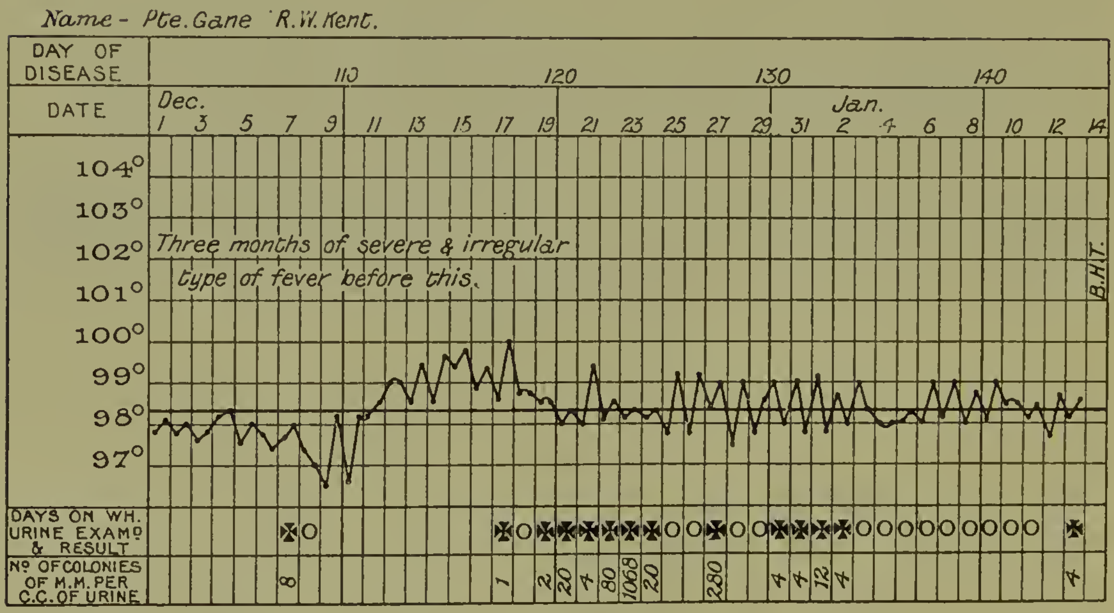


Chart 5.—Gane.

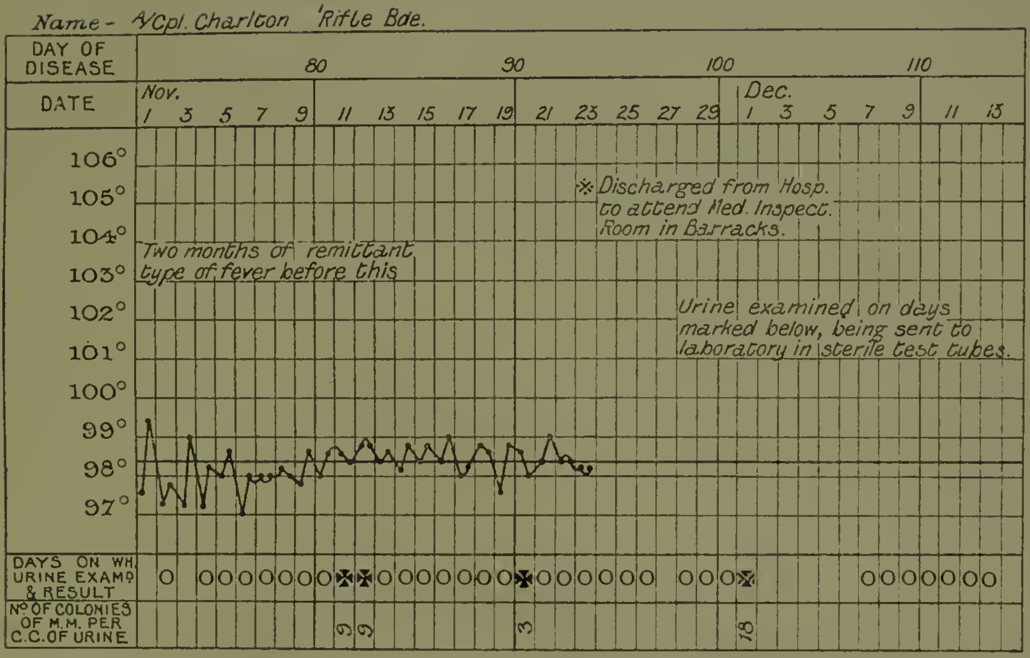


Chart 6.—Charlton.

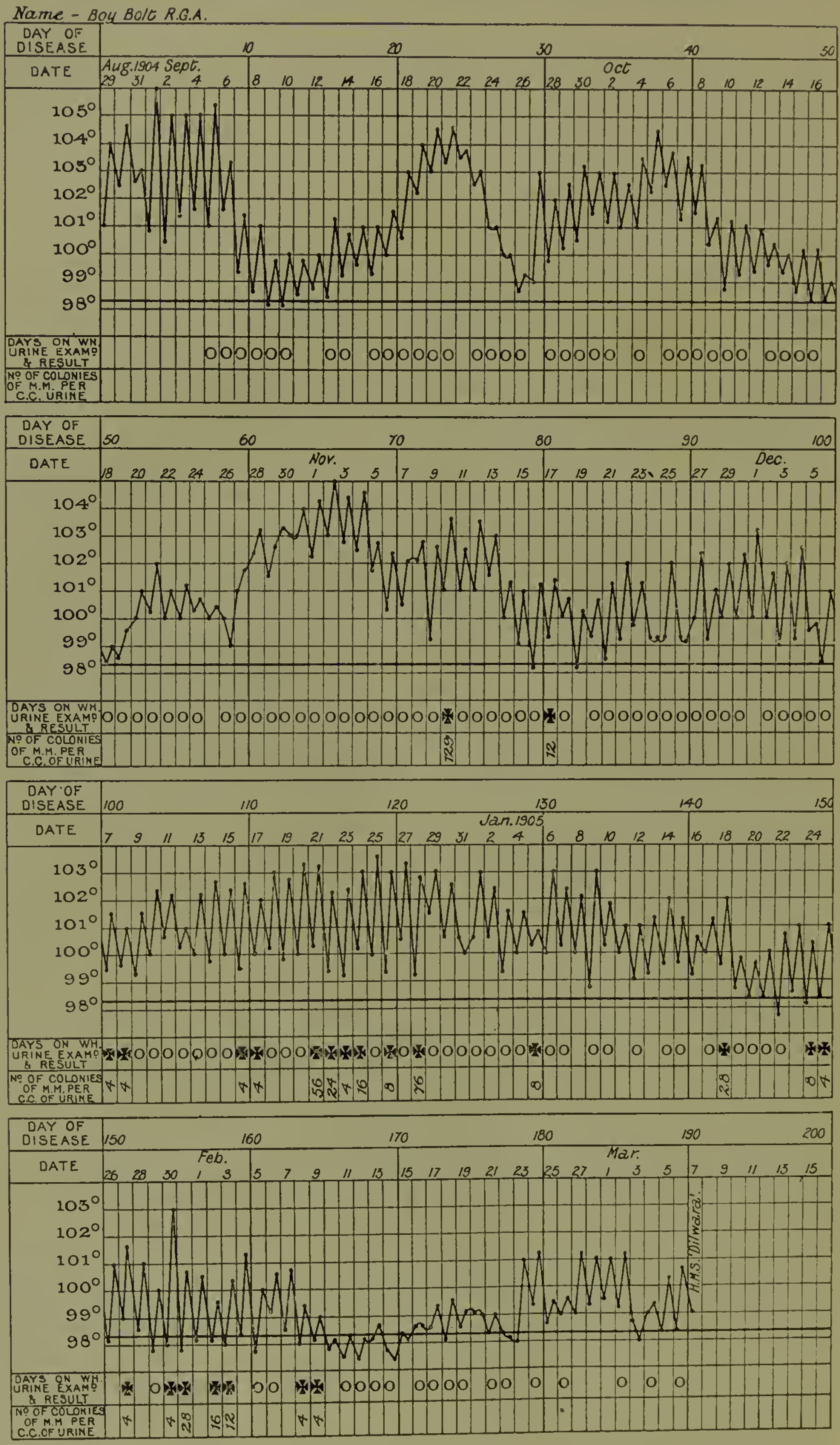
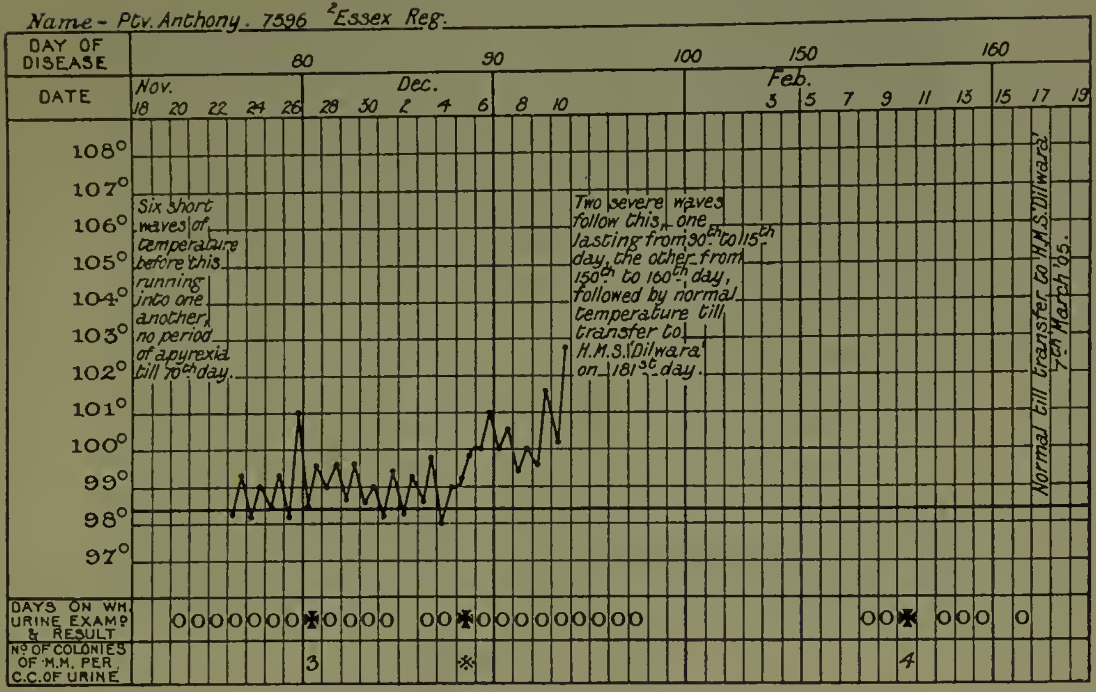
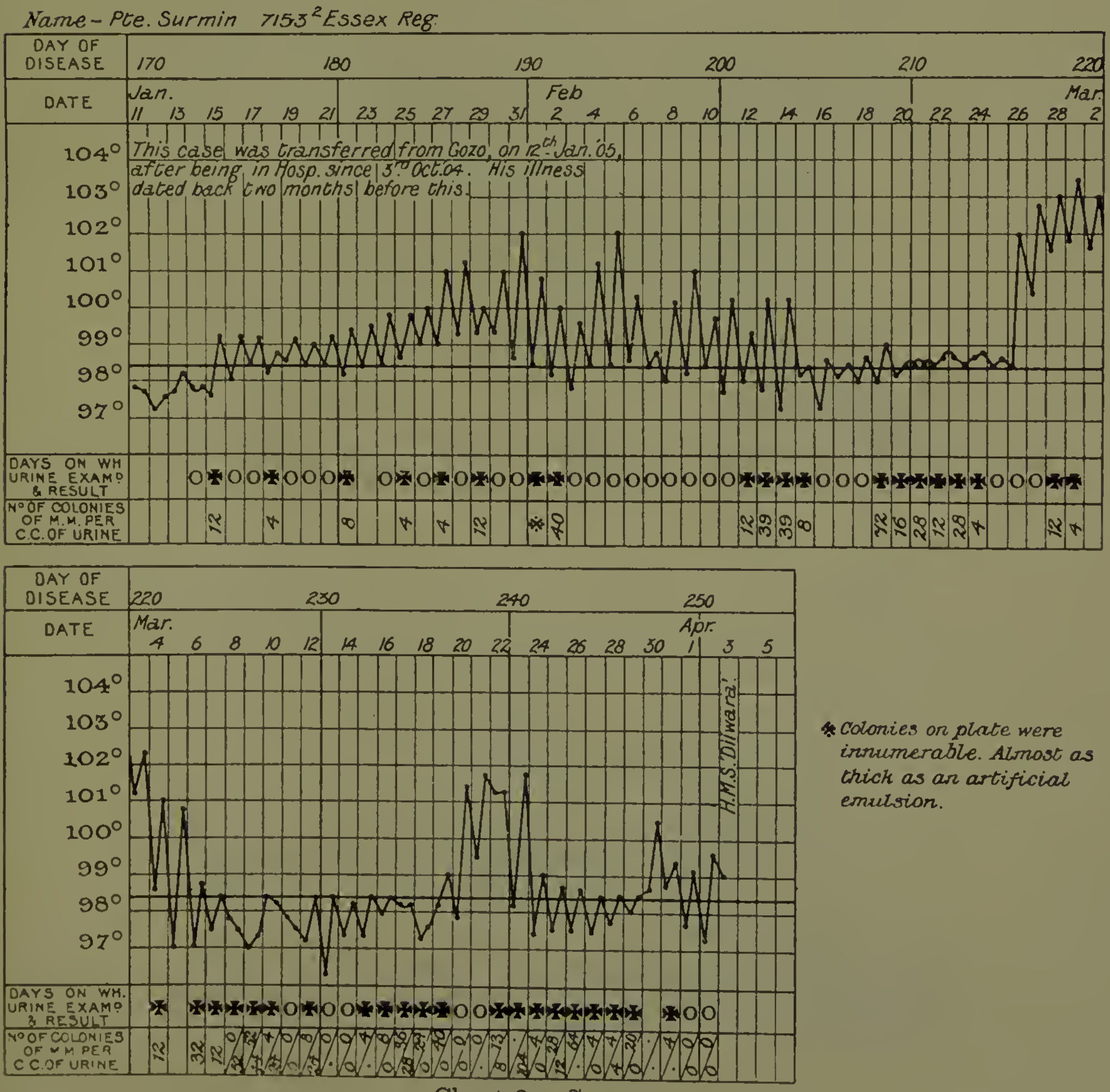

Name - Boy Bolt R.G.A.
DAY OF DISEASE
DATE
Aug. 1904 Sept.
Oct
Nov.
Dec.
Jan. 1905
Feb.
Mar.
105°
104°
103°
102°
101°
100°
99°
98°
DAYS ON WH. URINE EXAMº & RESULT
Nº OF COLONIES OF M.M. PER C.C. URINE
129
12
4
4
4
4
56
24
4
16
8
16
8
28
8
4
4
4
28
16
12
4
4
H.M.S. 'Dilwara.'

Chart 7.—Bolt.



※ Colonies absolutely innumerable, any number between 500 to 800 in each drop of urine.

Chart 8.—ANTHONY.



※ Colonies on plate were innumerable. Almost as thick as an artificial emulsion.

Chart 9.—SURMIN.

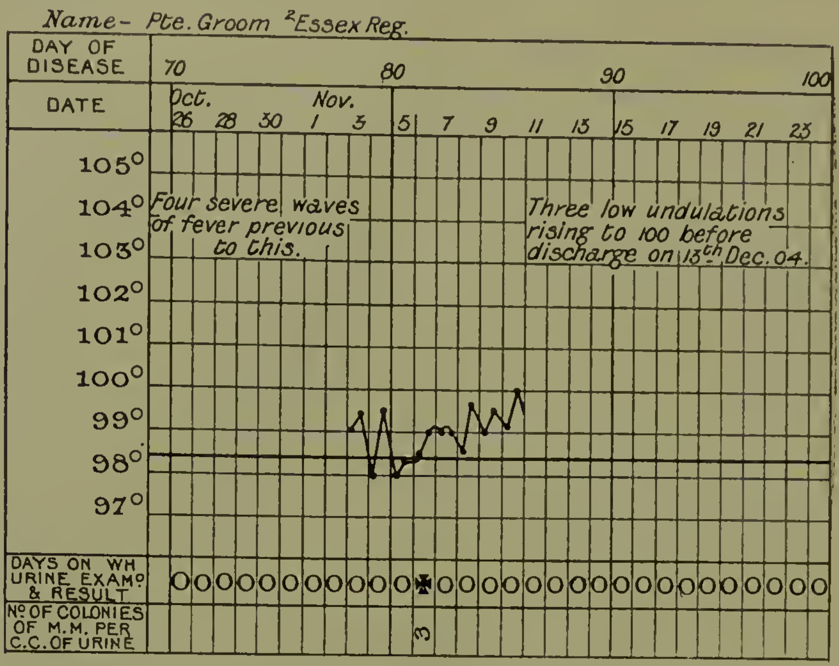


Chart 10.—Groom.

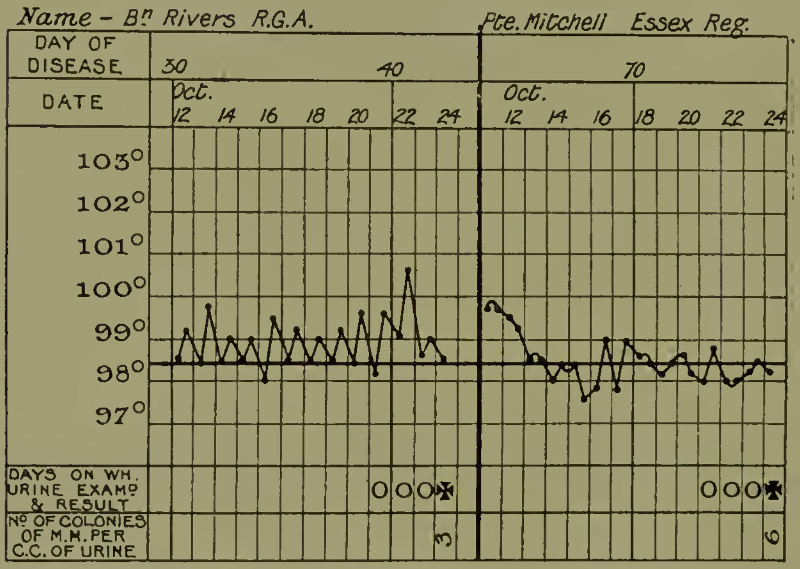


Chart 12.—Rivers.

Chart 11.—Mitchell.

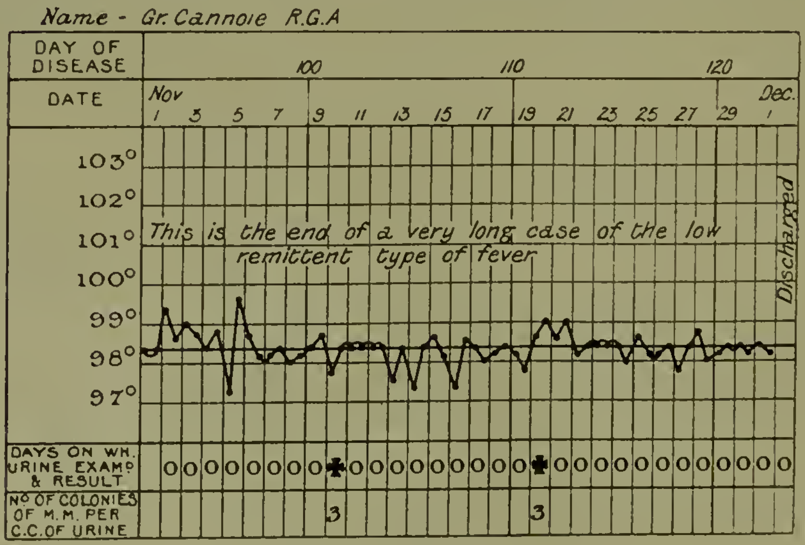


Chart 13.—Cannole.

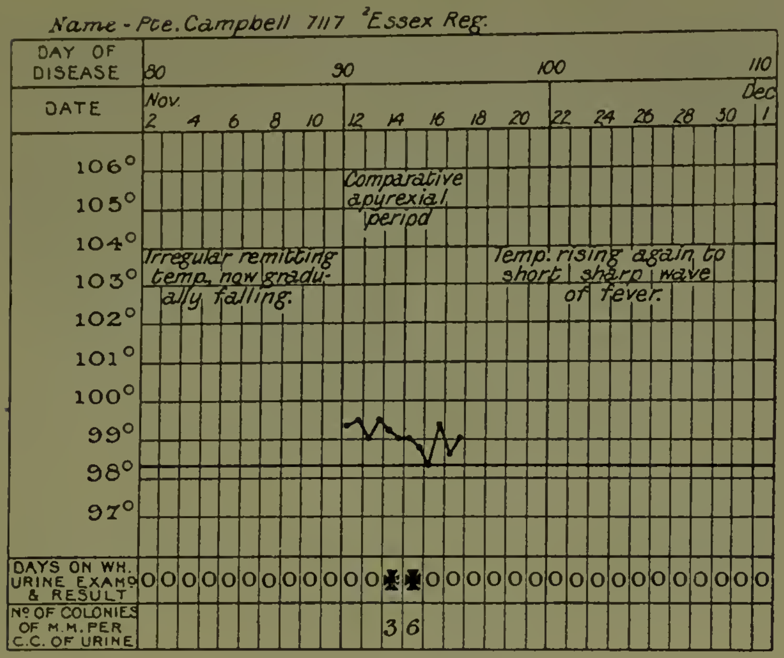


Chart 14.—Campbell.

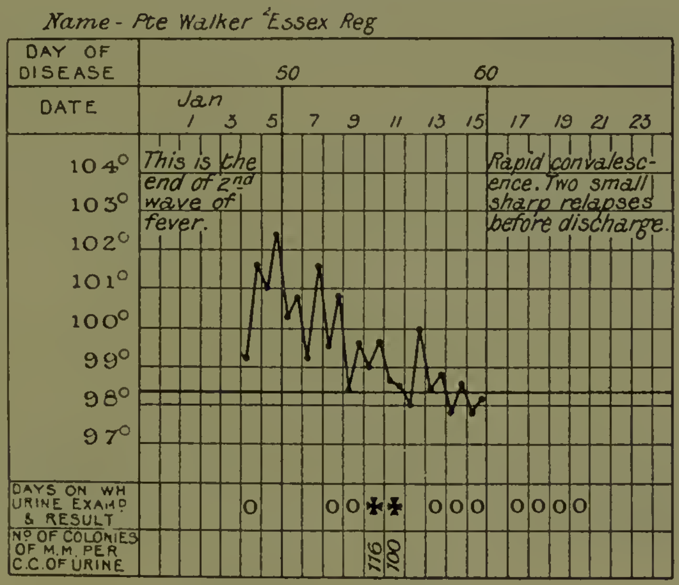


Chart 15.—Walker.

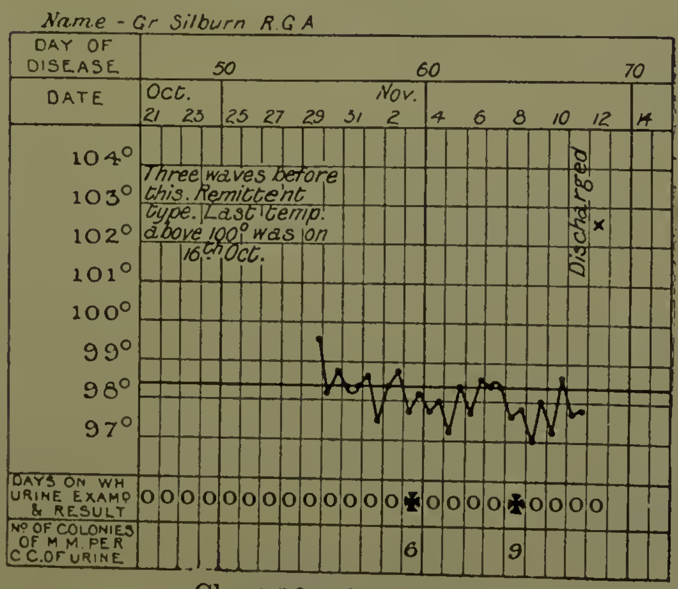


Chart 16.—Silburn.

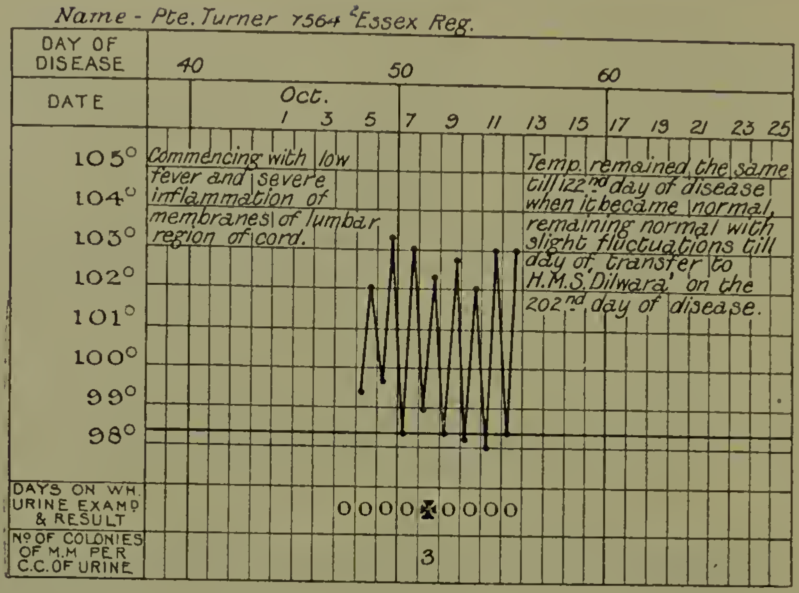


Chart 17.—Turner.

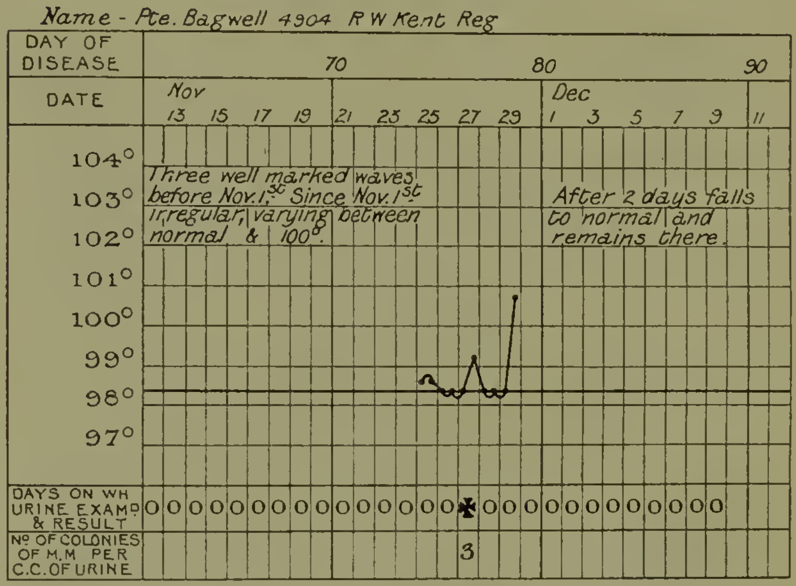


Chart 18.—Bagwell.

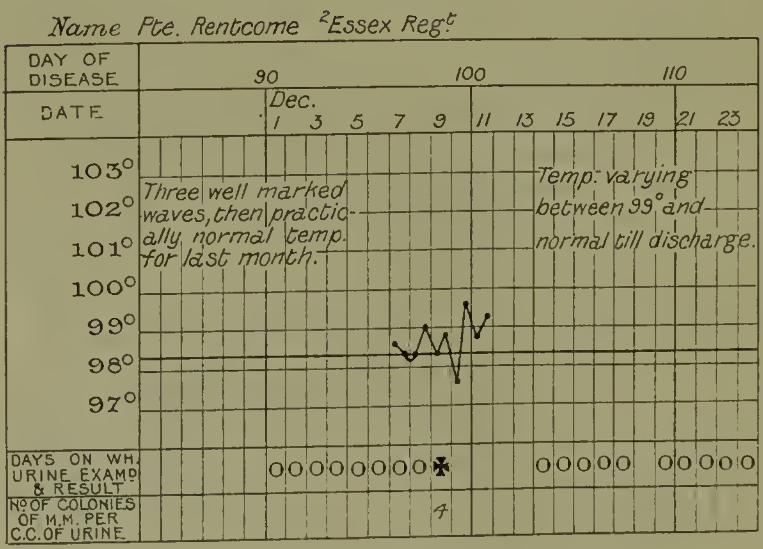


Chart 19.—Rentcome.

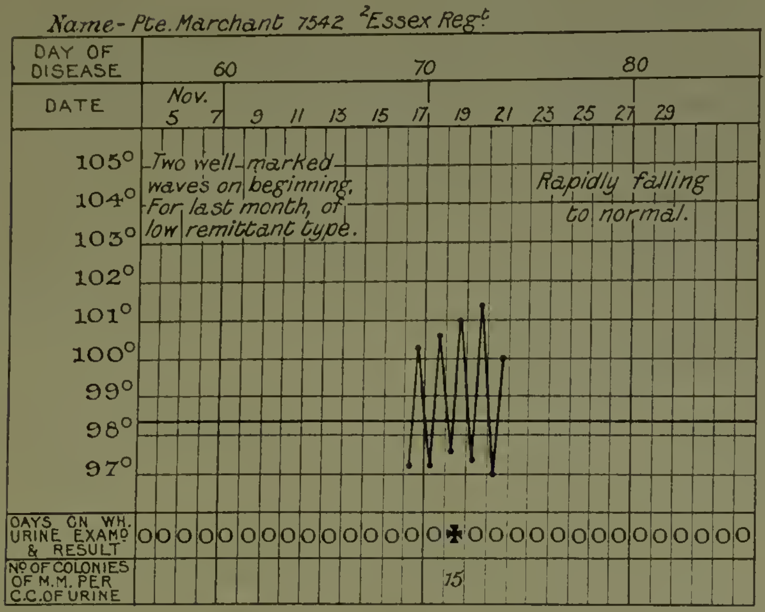


Chart 20.—Marchant.

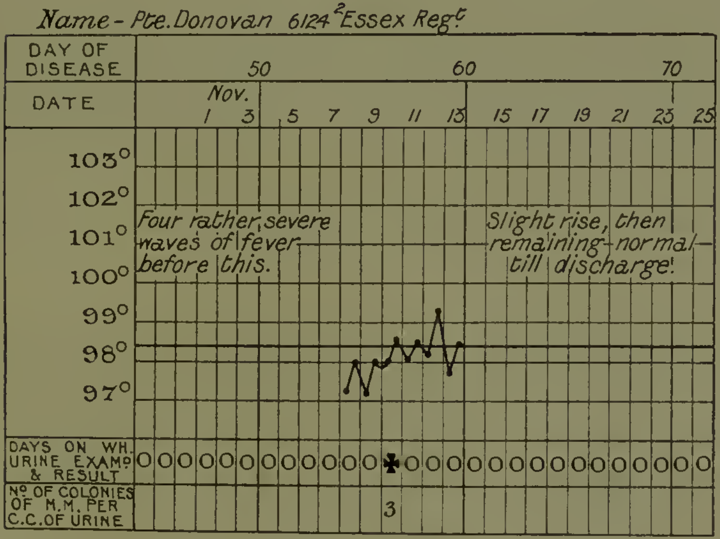


Chart 21.—Donovan.

Summary.—The excretion of *M. melitensis* in the urine would appear to be of two kinds :—

(1) A sudden enormous gush which stops as suddenly as it appears.

(2) A long continued excretion of small quantities.

As examples of the first see Cases Nos. 8 and 9.

As examples of the second see Cases Nos. 1, 2, 5, 7 and 9.

The period of disease most favoured is early convalescence or the last stages of the fever, especially just as a "wave" of fever is subsiding and the temperature reaching normal. It will be noticed that excretion tends to stop if another "wave" begins (see Charts 3, 8 and 9).

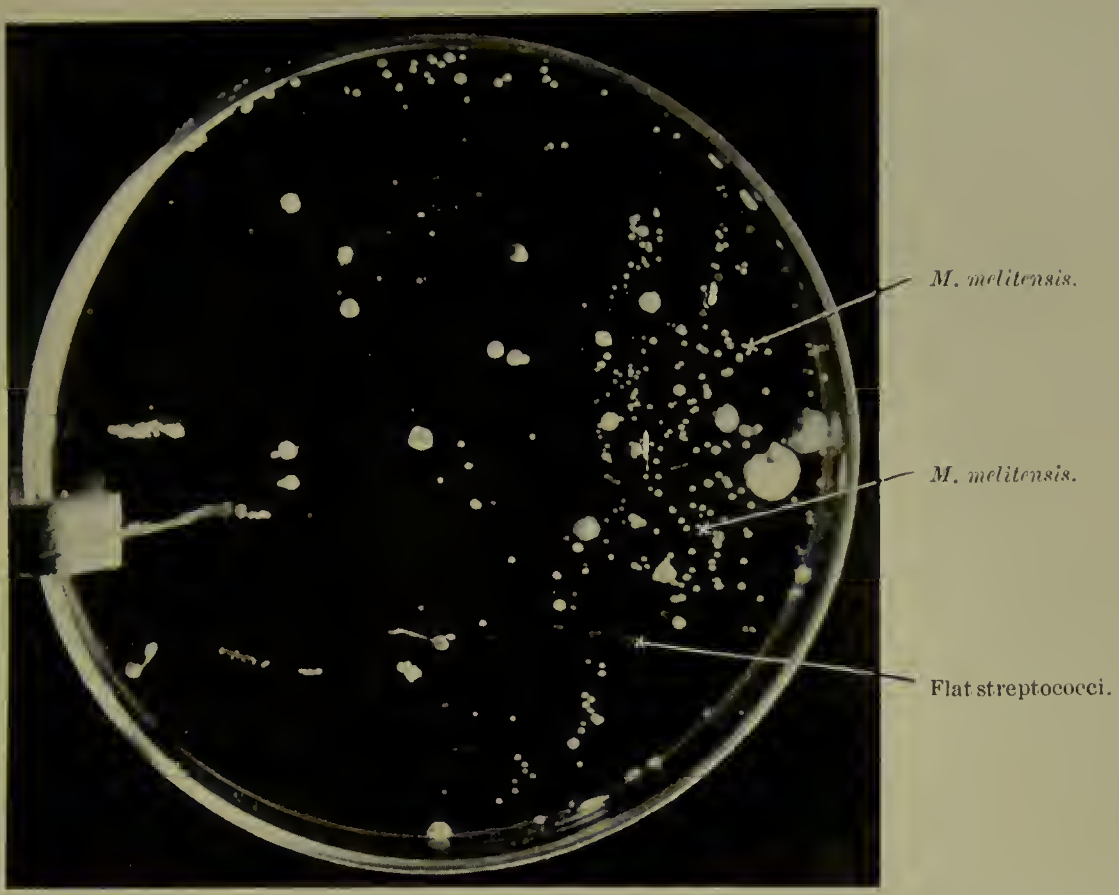
The time of day or rather the period of the 24 hours during which the excretion is greatest, appears to be between 6 P.M. and 6 A.M. Case No. 9 was for some time examined morning and evening, viz., the urine passed at 6 A.M. and that passed at 6 P.M. On 14 days on which the urine was examined at both these times *M. melitensis* was recovered, and taking the average daily excretion for the morning and evening it was found to be 20 colonies per cubic centimetre for the morning to 14 for the evening. On the 14 days mentioned above, *M. melitensis* was found 13 times in the morning urine and only eight times in the evening (see Chart).

The length of time after recovery during which the excretion may continue appears to be considerable, see Cases Nos. 3, 5 and 6. In the case of No. 6, the patient was discharged from hospital cured, but with orders to attend daily at the Medical Inspection room. I supplied him with sterile test-tubes and he daily supplied me with a fresh sample of his morning urine; on the seventh day after his discharge I found six colonies per $\frac{1}{5}$ c.c.

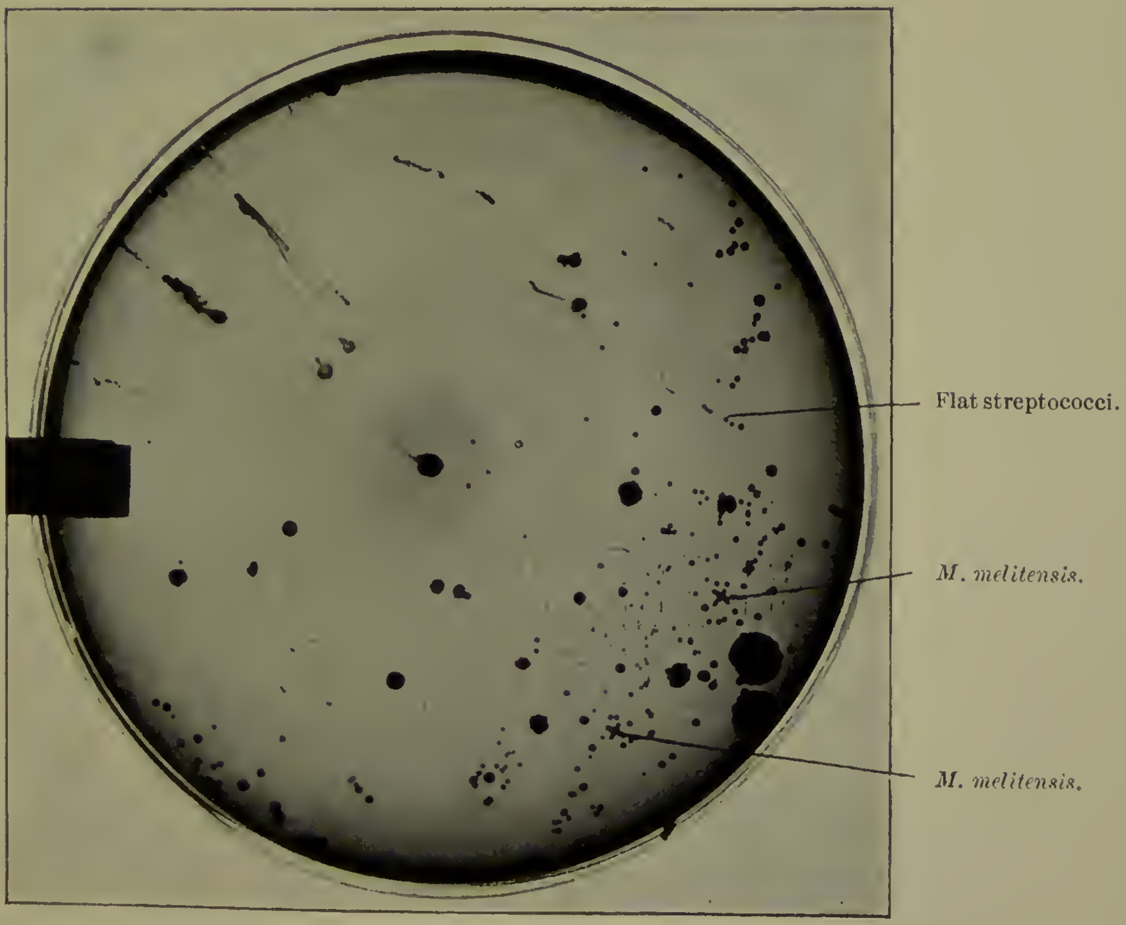
The longest period (temperature not above 99) after which excretion has been proved is 33 days; but see Case No. 1, where from the 66th day to the 111th day temperature never reached 100, yet *M. melitensis* was recovered all this time. There were, however, slight periodical rises to 99°·4, indicating the presence of infection in the system.

The most severe and prolonged cases were the ones that provided the most recoveries. The latest day of disease on which *M. melitensis* was recovered was the 249th.

I attach the photographs of a plate made by planting out $\frac{1}{4}$ c.c. of urine and incubating for four days. I tried to bring out the globular pearly white appearance of the colonies, and found it could best be done by using reflected light and a dark background. See also photographs of Surmin, February 1, further on.



Reflected light.



Direct light (same plate).

V. ON THE VITALITY OF *MICROCOCCUS MELITENSIS* IN URINE (in which it has been excreted), ON CLOTH, IN DUST, STERILE TAP WATER, AND STERILE MILK.

(Being Experiments 1 to 6, suggested by the Sub-Committee.)

By J. Crawford Kennedy, Capt. R.A.M.C., Member Mediterranean Fever Commission. Malta, April, 1905.

Experiments 1 *and* 2.

How long does the *M. melitensis* retain its vitality in urine?

The following procedure was adopted:—A batch of urines was collected every day in sterile test-tubes plugged with cotton wool, and after ¼ c.c. from each had been planted out on plates they were laid aside. The next day, and every day till the 4th day, ¼ c.c. was again planted out on Petrie dishes. On the 4th day the plates made on the 1st day were sufficiently incubated, and the presence or absence of *M. melitensis* was noted. Those urines from which *M. melitensis* was absent were then discarded.

It was soon found that *M. melitensis* could be recovered after four days in urine, so the urines were left undisturbed for the four days until it was determined which samples contained *M. melitensis*. Those samples were then plated out day after day, the more plates being used each day according to the length of time the urine had been kept.

As the majority of the samples contained *M. melitensis* in very small quantities, the urine was disturbed as little as possible, so that, supposing the *M. melitensis* had been found in one sample on the 5th day, the next sample of urine was not plated out till the 6th day, and so on. In this way 525 samples of urine were gone through. In 53 of these *M. melitensis* was recovered in the first instance, but in only 12 was it recovered a second time.

The following table (p. 72) shows at a glance the result of the examination of these 12 samples.

The *M. melitensis* has therefore been recovered from urine 16 days old. The points that favour its existence in urine are—

1. Acidity,
2. Absence of other organisms, whether acid or alkaline.

1. The urine of Mediterranean Fever patients is markedly acid, and if moderately free from contaminating germs will remain so for a

Date.	Name.	Number of colonies of *M. melitensis* found on 1st day per plate.	Result of examination of urine after standing the number of days indicated. + = *M. melitensis* recovered.																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Dec. 7	Kinsella ...	4	+	0	0	0	0	0	0																
17	Bolt.........	1		0	+	0	0	0	0																
20	Gane	5			+	+		0	0	0	0														
21	Gane	1			+		+	+	+	0	0	0	0												
	Bolt.........	14			+		0	0	0	0	0														
		A				+	0	0	0	0	0	0													
27	Gane	70 B						0	+	0	0	0													
		C							+	0	+	0			0										
31	Gane	1						+	+			+	+		0		0								
Jan. 1	Gane	3						+		0	0	0		+				+			0				
10	Walker ...	29 (alkaline)						+		0		0													
13	Gane	1													+	+	0	0	0	0					
Feb. 1	Surmin ...	—			+			0																	
13	Surmin ...	13																+						0	
			+	0	+	+	+	+	+	0	+	+	+	+	+	+	0	+	0	0	0			0	

very long time. Both the urines in which *M. melitensis* was recovered on the 16th day were acid on that day. The urine of Gane remained acid 29 days.

Two other samples (Kinsella, December 7 and 11, 1904) were tested daily for acidity to litmus; the sample of December 7 began to turn alkaline on January 27, and the other on January 26—*i.e.*, 51 and 46 days respectively. At the same time these samples, when planted out on plates, were found to be very filthy by the 5th or 6th day.

In only one sample was *M. melitensis* recovered, when the urine gave an alkaline reaction—viz., Walker sample, January 10. This urine was alkaline on the 1st day, and *M. melitensis* was recovered after it had stood for six days.

2. The presence of other organisms in the urine in any quantity is fatal to the recovery of *M. melitensis.* I am inclined to think that it may exist alongside the others, but that on nutrient media the excessive acidity or alkalinity produced by these rapid growing organisms prevents its development.

On several occasions I have found colonies of *M. melitensis* in a very acid plate hiding under the shelter (as it were) of a large alkali producing colony, where the acid is neutralised by the alkali.

Colonies that grow under these difficulties are always very tiny, do not have the amber colour by transmitted light, and in salt solution tend to remain in chains; the subcultures, however, are typical.

The sample of Surmin, February 1, was one of the two which contained *M. melitensis* in enormous quantities, and should have been an excellent sample for this experiment. *M. melitensis* was easily recovered from it on the 3rd day, but could not be isolated on the 6th, as the plate was overgrown with acid streptococci.

I attach a photograph (Plate 2) of the plates made from this sample on the 1st and on the 3rd day. It will be noticed that they are practically pure cultures of *M. melitensis.* The second plate is not so vigorous a growth as the first, the colonies tend to be smaller and less well defined, though their number is just as great as in the first. Both were incubated for four days. I have taken them in such a way that the light is reflected from the surface of the colonies, which thus stand out in relief.

Experiment with Artificially Infected Urine.

On December 16, 1904, the *M. melitensis* from the urine of a patient was added to the freshly passed urine of another, until the urine became cloudy. This was allowed to stand in a sterile test tube plugged with cotton-wool.

M. melitensis was easily recovered up to December 22 (six days). On the 23rd the growth of *M. melitensis* on the plate was beginning to get faint.

On the 24th the growth of *M. melitensis* was merely a faint blue haze along the edge of the track left by the drops of urine as they ran over the plate.

On the 26th *M. melitensis* could not be recovered as the plates were quite overgrown with acid streptococci. The urine remained acid for six days, after when the experiment was stopped.

Results.—The *M. melitensis* retains its vitality in urine (naturally infected) for 16 days, provided the urine remains acid and is fairly free from contaminating organisms.

It has been isolated from a urine (naturally infected) which had turned alkaline after six days.

In an artificially infected urine *M. melitensis* retained its vitality for seven days.

Experiments 3, 4, 5 *and* 6. *Suggested by the Sub-Committee.*

In carrying out these experiments, the great difficulty has been to know when one is dealing with a urine that contains the *M. melitensis.*

A urine collected one day and planted out on Petrie dishes has to be kept four days before the presence or absence of *M. melitensis* can be ascertained. By this time, even if *M. melitensis* be found in the plates, it is no guarantee that it has not died out by the 4th day, and in any case the vitality of any survivors has probably been seriously impaired.

To overcome this difficulty, I looked round for cases that would give a fairly regular supply of infected urine. In this I was fairly fortunate, and then my procedure was as follows:—

A sample of urine was collected every day from these patients, and every day it was—

1. Plated out on nutrose glucose litmus. This served as a control.
2. Allowed to dry on pieces of khaki drill.
3. Mixed with sterile dust.
4. Added to sterile tap water.
5. Added to sterile milk.

On the 3rd and 4th days the control plates were examined, and if *M. melitensis* was present the Experiments (2, 3, 4, 5) were proceeded with. This meant increasing the work greatly, as many samples proved useless.

The majority of samples treated in this way, and proved to be infected, contained *M. melitensis* in very small quantities—*i.e.*, 4 to 30 colonies per cubic centimetre urine. It will be readily understood that the chances of recovering it again after diluting the sample 100 times are very small.

I.

Experiment 4.

How long does *M. melitensis* in urine, when dried on cloth, retain its vitality?

A. A series of experiments was first made with urine artificially infected by *M. melitensis.* The method adopted was as follows :—

The cloth used was khaki drill, thoroughly sterilised and cut up into small pieces of ½ inch square. One-quarter of a cubic centimetre of the infected urine was then placed on each piece of cloth, and allowed to dry naturally. At varying intervals a piece was teased out in sterilised water, and the water planted out on a series of Petrie dishes containing the nutrient medium. This was found to be the best way of recovering the *M. melitensis*, as, if the urine-contaminated cloth was first treated in broth, the rapid-growing organisms would render its recovery impossible.

(1) January 8. *M. melitensis* recovered from urine added to Mediterranean Fever urine 24 hours old with acid reaction. Procedure as above, except that the cloths were put in the incubator for six hours at 37° C. to dry the quicker.

Series of plates were made on January 10, 12, 14, and 16 from pieces of the cloth. *M. melitensis* was not recovered on any of these days; the plates were found to be very acid, and overgrown.

On the 17th another piece was teased out in sterile distilled water, and phenol phthalein added as an indicator. It required two drops of *n*/10 alkaline solution to render the mixture alkaline to the indicator. Two more drops of the alkaline solution were added, and then the mixture plated out on a series of Petrie dishes. The result was that *one colony of* M. melitensis was found in one of the plates. This colony was put through the tests, and proved to be *M. melitensis.* This experiment was repeated with another cloth on January 23, but without success.

Result :—M. melitensis *found living in a very filthy cloth after nine days.*

(2) January 16. An experiment similar to the preceding was started, with this difference that the urine was fresh and it was allowed to dry naturally. A control plate was made from the infected urine, and the cloth examined on January 17, 18, and 23. The result in every case was nil, the plates all being very foul and acid.

(3) January 20. Another urine was artificially infected and the same procedure carried out. A control was made from the urine on the 1st day and *M. melitensis* was easily recovered.

The cloth was examined on the 21st and 25th *and* M. melitensis *was easily recovered* on these days.

In contrast to the other two experiments the plates were very clean and comparatively free from contaminations.

The cloth of the 21st provided *M. melitensis* in great quantities, that of the 25th rather scantily.

On February 1 another cloth was examined. No *M. melitensis* was recovered, the plates were very clean.

On February 3 the cloth gave a few colonies of *M. melitensis*, this was the 14th day of the experiment. I continued examining these cloths every other day but never found *M. melitensis* again; the plates were always very clean, so that there was no chance of its being hidden by other organisms as was the case in the two former experiments.

Result.—M. melitensis *found alive after* 14 *days.*

B. The carrying out of this experiment with a naturally infected urine was very difficult and disheartening; it was only after many failures that I obtained a suitable urine.

This was urine of Surmin, February 1; it was not one of the samples which I had been using in the series of cloth experiments and so had not been put on to cloth on the 1st day. But having found *M. melitensis* in great quantities after the plate had been incubating three days and having fortunately kept the sample, I was able to put it on cloth when it was three days old; at the same time I made a control plate from this urine, and by looking to the report dealing with the vitality of *M. melitensis* in the urine, the photographs of this control plate and the plate of the 1st day will be seen side by side, showing the presence of *M. melitensis* in enormous quantities.

February 4. Urine of Surmin, February 1, put on cloth. Control as above.

February 7. Cloth examined. M. melitensis *recovered* in great quantities.

February 17. Cloth examined. *No* M. melitensis recovered.

February 21. Cloth examined. *One colony of* M. melitensis *recovered.* This colony was of a very dark amber colour. It answered all the tests for *M. melitensis* perfectly. I continued for some days making plates from the cloth but got no further recovery, though everything was favourable, as the plates showed very little growth.

Result.—M. melitensis *excreted in urine dried on cloth will retain its vitality for* 17 *days*, though it tends to die out before the 13th day.

It should be remembered that this sample of urine had stood for three days before being put on cloth, so that the vitality of the *M. melitensis* had probably been impaired.

II.

Experiment 3.

How long does *M. melitensis* retain its vitality in dust moistened with infected urine?

Procedure.—Dust used was the dust and mud from the road, which, when dry, blows about as a very fine powder. It was thoroughly sterilised in hot air chamber. The infected urine was mixed up with it until it became of a pasty consistency. This was allowed to dry at room temperature and when dry was examined for *M. melitensis.* A small quantity of the dust was mixed up with sterile distilled water, thoroughly shaken, and the fluid pipetted off and planted out on plates. The dust generally was so fine that it would form an emulsion in the water and the whole mixture could be planted out. In this way it was found that *M. melitensis* could very readily be recovered.

A. A series of experiments was first performed with dust and urine which had been artificially infected with *M. melitensis* recovered from urine. The first two of these were unsuccessful owing to the very filthy state of the urine used. The third gave the following result:—

January 20. *M. melitensis* recovered from urine added to Mediterranean Fever urine; resulting emulsion added to dust which was allowed to dry at 16° C.

January 21. Small quantity plated out. M. melitensis *in great quantities* recovered on 3rd day, still more on the 4th.

January 25. Small quantity planted on one plate; same result as on 21st. M. melitensis *recovered.*

February 3. Another plate made. M. melitensis *recovered* in good quantities.

February 9 (20th day). Three plates made. Two *contained* M. melitensis in good quantity; one contained none. All the plates were very clean.

February 17 (28th day). Two plates made. M. melitensis *recovered* from both in great quantity.

February 25 (36 days). Two plates made. *No* M. melitensis was recovered from these plates.

March 1 (41 days). Six plates were made. M. melitensis *was recovered* from only two plates and in very small numbers.

March 4 (44 days). Seven plates made. Two of these *each contained one colony of* M. melitensis. This finished the supply of infected dust.

Result.—It is evident from this that the *M. melitensis retained its vitality* in the dust with no difficulty *for one month*, but after that time it died out quickly, but could be found when large quantities of dust were used and many plates made *up to the* 44*th day.*

B. Series of experiments were then made with urines naturally infected on the lines laid down in the beginning of this Report, viz., mixing consecutive series of fresh samples of urine with dust and then waiting until the controls were positive or negative for the presence of *M. melitensis.*

(1) In the first series *M. melitensis* was found in five controls, but only in quantities of 1 to 8 colonies per ¼ c.c. These dusts were plated out frequently and in large quantities, but no recovery of *M. melitensis* was made.

(2) In the second series 12 controls contained *M. melitensis*, but no *M. melitensis* was recovered from the dust. The number of colonies of *M. melitensis* in the controls of this series varied from 1 to 13 per ¼ c.c.

(3) On February 4 it was found that a sample of Surmin's urine passed on February 1 contained *M. melitensis* in large quantities (see Report on Vitality in Urine and Cloth Experiments), and some of it was mixed with dust and allowed to dry at room temperature.

February 5. Dust was dry and some was planted out on three plates. M. melitensis *was recovered* from each of these plates in great quantities, first appearing on the 3rd day.

Judging from the experiment with dust and artificially prepared urine, I allowed this dust to stand undisturbed till February 17 (13th day of experiment). On this day I again planted out some of the dust, but could not recover *M. melitensis.* I again planted it out on February 21, 25, and March 1, but could not recover it again.

Result.—Dust contaminated with naturally infected urine contained great *quantities of* M. melitensis *after* 24 *hours, but after* 13 *days the* M. melitensis *had completely died out.*

Note.—The same remark applies here as in the cloth experiment, viz., that the urine was three days old before being put in dust. Consequently, the vitality of *M. melitensis* may have been considerably impaired.

Experiments with Unsterilised Soil.

A red soil obtained from a garden bed was used. The method adopted was the same as for sterilised dust, but the proportion of sterile water to soil was greater. The urines used were the same as used in the second series of dust experiments. Plates were obtained in which the colonies were fairly discrete, but no *M. melitensis* was recovered.

III.

Experiment 5.

To determine vitality of *M. melitensis* in infected urine when added to sterile tap water. The method of procedure was as indicated above.

Fresh samples of urine were added to the sterile water contained in flasks of 100 c.c., or in test-tubes of 10 c.c.

One c.c. of urine was added to 100 c.c. and ¼ c.c. to 10 c.c. Controls were at the same time taken from each sample of urine and if *M. melitensis* was found the experiment was proceeded with.

In this way 20 samples of urine were tried, five in flasks and 15 in test-tubes.

Of those put in flasks three were found to contain *M. melitensis* in small quantities (viz., 72, 8, and 4 colonies per cubic centimetre). These three flasks were plated out on 4th, 7th, 8th, 14th, 15th, 17th, and 25th days. *M. melitensis* was not recovered.

Of those put in test-tubes, two were found to contain *M. melitensis* (4 and 12 per cubic centimetre). These were plated out on the 5th, 8th, 12th, and 15th days. *M. melitensis* was not recovered.

Being so unsuccessful with the experiment conducted with naturally infected urine, I then carried it out with urine artificially infected with *M. melitensis.*

February 14. The urine used was that of a Mediterranean Fever patient, freshly drawn, and the *M. melitensis* used had also been isolated from the urine of a Mediterranean Fever case.

The *M. melitensis* was added to the urine until a milkiness was visible. One c.c. of this emulsion was added to a flask containing 100 c.c. of sterile tap water and the flasks allowed to stand in the laboratory cupboard, where there was practically a constant temperature of 15° C.

On the following days, $\frac{1}{4}$ c.c. of the sediment was plated out on Petries containing nutrose-glucose-litmus-agar, February 15, 16, 17, 18, 19, 21, 23 and every day to March 4, 6, 11, 15. M. melitensis *was recovered up to and on March* 6, *being the* 20*th day of the experiment.*

For the first four days only one plate was taken from the flask, and the result was as follows:—On February 15 (the first completed day) the *M. melitensis* was recovered in great quantity, the first appearance being noticed on the 17th (48 hours' growth); on February 16 only five colonies appeared; on February 17 one only, and on the 18th none. Thereupon three plates were made each day and *M. melitensis* was always recovered from one or two of them.

The plates taken from February 21 to March 11 all contained numbers of colonies which were practically identical with *M. melitensis* to the naked eye by transmitted light, but by reflected light were a dull white and opaque instead of having a greenish blue halo appearance. This organism was a coccus slightly larger than *M. melitensis,* and which tended to remain in chains of four and five when emulsified in salt solution.

This organism gave a great deal of bother, especially towards the end of the experiment, as I found it increasingly difficult to separate the *M. melitensis* from it. When fishing a *M. melitensis* colony from the plate, and feeling certain that nothing else had been touched, I found that the sub-culture was more frequently than not contaminated by this organism, which in the sub-culture on nutrose-glucose-litmus grew with the production of acid. It was generally necessary to sub-culture twice before obtaining a pure growth of *M. melitensis.*

This organism when sub-cultured was an acid producing streptococcus not agglutinated by Mediterranean Fever serum and partly losing its stain when treated by Gram's method.

On March 11 and 15 no *M. melitensis* was recovered and the plates made on the latter date were very dirty, being overgrown with rapid growing alkaline colonies.

Conclusions. That *M. melitensis tends to die out quickly in sterile tap water, but can be recovered from it up to the* 20*th day.*

Experiment 6.

To determine survival of *M. melitensis* in infected urine which has been added to sterile milk. This experiment was carried out on the same lines as the former. In every case test-tubes containing 10 c.c. of sterile milk, to which a little litmus had been added, were used; the amount of urine added to each test-tube of milk was ¼ c.c.

December 12. Six samples of urine added to six litmus milk tubes. Control: no *M. melitensis* found in any one of the samples.

December 13. Five samples urine added to milk. Control: one sample (Kinsella) contained one colony *M. melitensis* per cubic centimetre. Four ¼ c.c. of the mixture of milk and this infected sample were plated out on four nutrose-glucose-litmus-agar plates on December 18, 24, 27. No *M. melitensis* was recovered.

December 14. Six samples urine added to milk. Control: one sample (Kinsella) contained two colonies *M. melitensis* per cubic centimetre. The infected milk was plated out on December 21, 24, and 27. No *M. melitensis* recovered.

December 19. Three samples urine added to milk. Control: none contained *M. melitensis.*

December 20. Ditto. Five samples of urine.

December 21. One sample urine from Boy Bolt. Control found to contain 56 colonies *M. melitensis* per cubic centimetre. The milk was plated out on the following days: December 27 (6th day of experiment). Milk showed no coagulation and remained alkaline. M. melitensis *recovered.*

December 31 (10th day of experiment). Milk still alkaline, no coagulation. M. melitensis *recovered.*

January 4 (14th day) }
January 8 (18th day) } Milk alkaline, *M. melitensis* not recovered.

January 12 (22nd day). Milk turned acid, *M. melitensis* not recovered.

December 24. Five samples of urine added to milk. Control: Bolt found to contain 16, and Gane 20 colonies *M. melitensis* per cubic centimetre. Bolt had turned the milk acid on the 31st. *M. melitensis* not recovered. Gane remained alkaline till January 4, *M. melitensis* not recovered.

December 24. Four samples urine added to milk. Control: one sample (Gane) was found to contain 280 colonies *M. melitensis* per cubic centimetre. The milk was plated out on the following days:

December 31 (4th day)
January 4 (8th „)
„ 8 (12th „)
„ 12 (16th „)
Milk remained alkaline all this time. M. melitensis *was recovered on each of these days.*

January 16 (20th day)
„ 20 (24th „)
„ 24 (28th „)
By the 20th day (January 16) milk had turned acid. No *M. melitensis* was recovered after this.

After having recovered the *M. melitensis* up to the 16th day, it was decided to carry on the experiment without touching the infected milk till the 17th day of the experiment.

January 21. One sample of urine added to milk. Control gave no *M. melitensis.*

January 22. One sample of urine, ditto.

January 24. One sample urine added to milk. Control contained eight colonies *M. melitensis* per cubic centimetre. Milk plated on following days:—

February 10 (17th day of experiment)
„ 14 (21st „ „)
Milk remained alkaline. *M. melitensis* not recovered.
„ 18 (25th „ „). Milk turned acid. *M. melitensis* not recovered.

January 25. One sample of urine added to milk. Control contains four colonies per cubic centimetre. Plated out February 12 and 18. *M. melitensis* not recovered.

January 27. Same as January 25. Plated out on February 14, *M. melitensis* not recovered.

January 29. One sample urine. Control contained no *M. melitensis.*

January 30. Same as January 25. Plated out February 16 and 22. No *M. melitensis* recovered.

Six more samples were added to milk in the same way on February 4, 5, 6, and 7, but the controls were negative.

The *M. melitensis* recovered above was proved by the usual tests.

The only thing to be noted as regards cultural or other appearance is that in plates of the 12th and 16th days the colonies were of a darker amber colour than usual.

Summary.

Forty-eight samples of urine were added to milk; control experiments proved the presence of *M. melitensis* in 10 of them. M. melitensis *was recovered* from two of these; in one up to the 10th day and in the other *up to the 16th day.* In both cases the milk remained alkaline as long as the *M. melitensis* was recovered.

These urines must have been very clean and comparatively free from acid organisms. In most cases the milk became curdled in four days' time.

Conclusions.—M. melitensis will live in sterile milk which has been contaminated by infected urine as long as the milk remains alkaline or neutral to litmus.

Summary.

M. melitensis in the condition in which it is excreted in urine will retain its vitality—

1. Dried on cloth for 17 days.
2. In dust: for less than 13 days.
3. In sterile tap water. Not recovered.
4. In sterile milk for 16 days.

M. melitensis derived from urine, grown on media and then added to Mediterranean Fever urine, retains its vitality—

1. Dried on cloth for 14 days.
2. Dried in dust for 44 days.
3. Mixed with sterile tap water for 20 days.

VI. A PRELIMINARY NOTE ON THE EXAMINATION OF THE BLOOD OF GOATS SUFFERING FROM MEDITERRANEAN FEVER.

By Dr. T. Zammit, Member of the Mediterranean Fever Commission.

On June 14, as detailed by Major Horrocks, I examined the blood of six goats, which were brought to the lazaretto on June 12, and obtained the following results :—

Goat No. 1.—Strong immediate reaction, in dilution of 1 to 20.
Goat No. 2.—Strong immediate reaction, in dilution of 1 to 20.
Goat No. 3.—Strong reaction, after half-an-hour.
Goat No. 4.—No reaction.
Goat No. 5.—Strong reaction, after half-an-hour.
Goat No. 6.—Strong immediate reaction.

On June 15 the bloods were again examined, with identical results.

On June 18 about 5 c.c. of blood were taken from Goat No. 6, and distributed in six broth-tubes. On June 25 passages from the broth-tubes were made on to agar slopes, and the *M. melitensis* recovered in pure culture. This micro-organism was also recovered from the blood of Goat No. 5.

Blood has also been taken from Goats Nos. 1, 2, and 3, and distributed in broth-tubes as usual, but, so far, the *M. melitensis* has not been recovered.

Material from Abattoir.—Dr. Caruana Scicluna having suggested that possibly infected goats might be met with in the abattoir, I have examined 46 spleens removed with aseptic precautions, but, so far, have only recovered the *M. melitensis* from one. The blood from the goats was examined for agglutination with the *M. melitensis*, and a definite reaction was obtained in seven.

(For further details, see 'Proceedings of the Royal Society,' Series B, vol. 76, 1905, No. B 510.)

VII. PRELIMINARY NOTE ON GOATS AS A MEANS OF PROPAGATION OF MEDITERRANEAN FEVER.

By Major W. H. Horrocks, R.A.M.C., Member of the Mediterranean Fever Commission.

(Reprinted from the 'Proceedings of the Royal Society,' Series B, vol. 76, 1905, No. B 510.)

With the object of ascertaining, by experimental inoculation, whether goats could be infected by the *M. melitensis*, six goats were bought on June 12, 1905, from two different herds, and placed in the lazaretto. On June 14 Dr. Zammit, as a preliminary step to our experimental work, took blood from each of these goats, and proceeded to test the action of the serum on the *M. melitensis*. He found, to his great surprise, that the serum of five of the goats, when considerably diluted, caused agglutination of this microbe. On June 15 similar results being again obtained, Dr. Zammit brought specimens of the bloods to the Public Health Laboratory, and asked me to confirm his observations. I obtained the following results:—

Goat No. 1.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis*, visible to the naked eye. When diluted 1 to 100, however, the serum gave no reaction.

Goat No. 2.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis*. A dilution of 1 to 100 produced a complete reaction after 15 minutes.

Goat No. 3.—Blood serum diluted 1 to 10, 1 to 40, and 1 to 100, caused immediate agglutination of the *M. melitensis*, but, in the case of the dilution 1 to 100, the clumps were not visible to the naked eye until after 15 minutes.

Goat No. 4.—The blood serum produced no reaction with the *M. melitensis*.

Goat No. 5.—The blood serum diluted 1 to 10 caused immediate agglutination, but dilutions of 1 to 40 and 1 to 100 did not produce a complete reaction until after 15 minutes.

Goat No. 6.—Blood serum diluted 1 to 300 caused complete agglutination of the *M. melitensis*, visible at once with the naked eye.

The reactions thus obtained, and especially that of Goat No. 6, suggested that possibly five of the goats were suffering from Mediterranean Fever, acquired under natural conditions. The goats were stated to be healthy, but were sold cheaply, as they had given very little milk for some time. They were bought from pens in the neighbourhood of Birchircara and St. Julians, and taken straight to the lazaretto, where they were placed in clean stalls, which had never been used for any experimental work with the *M. melitensis.*

Dr. Zammit and I then arranged to make a complete study of these animals; Dr. Zammit undertook the investigation of the blood, and I made myself responsible for the bacteriological examination of the milk and urine.

Bacteriological Examination of Milk and Urine obtained from Naturally Infected Goats.

Goat No. 6.—I commenced work with this goat, as its blood serum, when diluted 1 to 300, caused immediate agglutination of the *M. melitensis.* The animal did not appear well, and had a very poor coat. The udders were flaccid, but the milk exuded appeared normal in character. The temperature was taken morning and evening, and compared with that of a healthy goat. The evening temperature never rose above 103°, and, as this temperature is often recorded in the case of perfectly normal goats, a febrile temperature could not be said to be present. On June 18 milk was withdrawn, and 1 c.c. centrifugalised; the deposit was then carefully spread over 10 litmus-nutrose-agar plates. After four days' incubation at 37° C., colonies of the *M. melitensis* appeared in every plate. The colonies were at once tested with a dilute (1 to 100) specific serum obtained from an inoculated rabbit. The micrococci were found to agglutinate at once, the clumps being visible to the naked eye. Some of the colonies were then planted out on agar slopes, and the resulting growths, when subjected to the usual confirmatory tests, showed that the *M. melitensis* was undoubtedly being excreted in the milk of this goat.

On June 22 the milk was again examined and the *M. melitensis* recovered once more.

On June 23 examination of the urine was commenced. The vagina was washed out with an antiseptic solution and a catheter, previously sterilised in boiling water, passed into the bladder. The urine so obtained was plated on litmus-nutrose-agar, but after four days' incubation at 37° C., in spite of the precautions taken, the plates were found densely crowded with saprophytic organisms, and the *M. melitensis* could not be detected.

On June 24 and 26 the urine was again plated, the same precautions being used, but the plates were densely crowded with foreign organisms and the *M. melitensis* could not be seen.

On June 27, 28, 29, and 30, and on July 1, 3, 4, 5, 7, 8, 9, and 10, the urine was also examined, but up to the present the *M. melitensis* has not been recovered.

The milk was plated again in June and July, and the *M. melitensis* was found on each occasion.

Result.—The *M. melitensis* appears to be steadily excreted in the apparently normal milk of this goat, but up to the present it has not been found in the urine.

Goat No. 1.—This animal appeared healthy, but the udders were flaccid, and the milk exuded had a thin serous appearance. The temperature was taken regularly, but no indications of fever were observed.

On June 22, 1 c.c. of the milk was centrifugalised and the deposit plated. After four days' incubation at 37° C., the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were obtained.

On June 26, 29, and 30 the urine was examined, but no signs of the *M. melitensis* could be discovered.

On July 1, 10 c.c. of the urine were centrifugalised and the deposit plated; four days later every plate was found studded with colonies of the specific microbe. The colonies were fished, planted on agar slopes, and the resulting growths tested in the usual manner.

Result.—The *M. melitensis* is excreted in very large numbers in the serous-looking milk of this goat. It is also excreted in the urine.

Goat No. 2.—This goat appeared quite well, and the milk exuded from the udders had a normal appearance. There were no indications of fever.

On June 22, 1 c.c. was centrifugalised and the deposit plated. After four days' incubation about 30 colonies appeared in every plate. On June 24 and 26 the milk was again examined, and colonies of the *M. melitensis* were recovered on both occasions.

The urine was examined on June 23, 26, 27, 28, 29, and 30, and on July 1, 3, and 6, but the *M. melitensis* could not be detected.

Result.—The *M. melitensis* appears to be excreted in small quantity in the normal-looking milk of this goat. It has not yet been detected in the urine.

Goat No. 3.—This goat looked healthy and had no fever, but its milk was thin and serous. On June 22 the milk was examined, one loopful of the serous milk being spread over each plate. After four days' incubation all the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were again obtained.

The urine was examined on June 23, 26, 28, and 30, and on July 1, 3, 6, 8, 9, 10, 11, but no signs of the *M. melitensis* could be discovered.

Result.—The *M. melitensis* appears to be present in enormous quantities in the thin serous-looking milk of this goat, but it has not yet been found in the urine.

Goat No. 5.—This goat was in poor condition, the udders were flaccid, and the milk exuded had a thick jelly-like appearance.

On June 22 the milk was examined, one loopful of the jelly-like material being spread over each plate. After four days incubation all the plates were covered with minute colonies of the *M. melitensis.* On June 24 and 26 the milk was again examined, and densely crowded plates were obtained as before.

On June 25 and 30 the urine was examined, but no colonies of the *M. melitensis* were detected.

On July 1 the urine was again plated, and four days later every plate was found to contain numerous transparent colonies strongly resembling those of the *M. melitensis.* Some of the colonies were fished and planted out on agar slopes. The resulting growths were then subjected to the usual confirmatory tests, and the *M. melitensis* proved to be undoubtedly present.

The five goats just examined being considered by their owners to be "out of milk," would not be likely to be employed for milking purposes, though in the case of Goats Nos. 2 and 6, the milk might easily have been used without any fear of suspicion arising as to its being abnormal. Consequently it appeared very desirable to examine the herds which were actually supplying milk to Valetta, Sliema, and the various hospitals.

I therefore asked Captain Kennedy, R.A.M.C., to visit the various herds, and, with the owners' consent, take blood from the ears, and test the action of the sera on the *M. melitensis.* The results he obtained are given in Part VIII; it will be seen that, out of 161 goats examined, 84 gave a reaction, corresponding to a percentage of 52 probably infected with Mediterranean Fever. I then obtained samples of milk from some of the apparently infected animals, and proceeded to plate them on litmus-nutrose-agar. The following results have been obtained up to the present time :—

Examination of the Goats supplying Milk to Forrest Hospital.

I visited this herd, which assembles outside the hospital gate every morning, and selected Goats Nos. 38, 48, 37, and 43 from Captain Kennedy's list.

Goat No. 38.—The milk from this animal was centrifugalised, and the deposit plated on July 4, 5, 6, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Goat No. 48.—The milk was examined on the same dates as Goat No. 38, but, so far, the *M. melitensis* has not been isolated.

Goat No. 37.—The milk of this animal was taken on July 4, and 2 c.c. centrifugalised; the deposit was then plated. After four days' incubation every plate was found densely crowded with small colonies of the *M. melitensis;* the colonies were so numerous that it was impossible to make an accurate count. The colonies were fished and planted on agar, the growths resulting responded to all the tests characteristic of the *M. melitensis.*

On July 5 and 6 the milk was again plated, and similar results were obtained.

As this goat was in full milk, there cannot be any doubt that the *M. melitensis* was being excreted in large numbers. A pint of the milk was then collected, and Dr. Zammit very kindly made a chemical examination of the sample. The result given below shows that the milk was of good quality.

Analysis of Milk from Goat No. 37.

Density at 15° C.	1030
Fat	4·3 per cent.
Total solids	13·18 ,,
Solids, non-fat	8·8 ,,
Ash	0·51 ,,

Goat No. 43.—The milk of this goat was examined on July 4, 5, 6, 7, 8, 9, and 10, but, up to the present, the *M. melitensis* has not been isolated.

A reference to Captain Kennedy's list shows that, while Goat No. 37 reacted in a dilution of 1 to 60, Goats Nos. 38, 48, and 43 only reacted in a dilution of 1 to 20, and were probably in an early stage of the disease.

Examination of a Small Herd supplying Milk to Valetta Station Hospital.

Goats Nos. 27, 30, and 32 were selected from this herd. The goats were kept at Casal Curmi, and brought every morning to the Station Hospital.

Goat No. 30.—On June 29 and 30 milk was centrifugalised and plated in the usual manner, but the *M. melitensis* was not detected.

On July 1 plates were again made, and a few typical colonies appeared.

On July 3 10 c.c. of the milk were centrifugalised, and the deposit plated; four days later every plate was found densely crowded with colonies of the *M. melitensis.*

On July 6 similar results were obtained.

A sample of the milk was then analysed by Dr. Zammit, and found to have an average chemical composition.

Goats Nos. 27 and 32.—The milk from these goats was examined on June 29 and 30, and on July 1, 3, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Examination of a Small Herd Supplying Milk to Valetta.

This herd assembled in St. John's ditch, and 17 out of 25 animals showed a blood reaction with the *M. melitensis*, and six of them reacted when the serum was diluted 1 to 100. Goats Nos. 50 and 52 were selected from Captain Kennedy's list.

Goat No. 50.—On July 6, 1 c.c. of the milk was centrifugalised and the deposit spread over the usual plates. Four days later all the plates were found densely crowded with small colonies of *M. melitensis.*

The confirmatory tests were applied in the usual manner. This animal was considered one of the best milkers in the herd, and its owner valued it at £5, whereas the ordinary price for a goat in milk varies from £3 to £4.

Goat No. 52.—This animal appeared in good health and its udders were full of milk. It was purchased and placed in the lazaretto.

On July 5 milk was withdrawn and 1 c.c. centrifugalised; the deposit was then spread over nutrose-agar plates in the usual manner. After four days' incubation at 37° C., all the plates were found so crowded with colonies of *M. melitensis* that a reliable count could not be made.

On July 6 and 8 the milk was again examined and similar results were obtained.

A sample of the milk was submitted to Dr. Zammit for chemical analysis; he obtained the following results:—

Specific gravity at 15° C........................... 1031
Total solids, 14·0 per cent.; fat, 3·6 per cent.; ash, 0·73 per cent.

Examination of a Herd Supplying Milk to Sliema.

Two goats were bought from this herd and placed in the lazaretto. The pens were in the neighbourhood of Misida.

Goat No. 15.—On July 5 the blood was examined and the serum, diluted 1 to 50, was found to cause complete agglutination of the *M. melitensis* visible to the naked eye. The goat appeared to be in good health, and the udders were full of milk. Some milk was withdrawn and 2 c.c. centrifugalised; the deposit was then plated in the usual manner. On July 9 the plates were found covered with small colonies of the *M. melitensis.*

On July 6 the milk was again examined, and the deposit from

1 c.c. produced as before an immense number of colonies of *M. melitensis.*

The urine was withdrawn by a catheter and plated on July 5, 6, 7, 8, 9, and 10, but up to the present the *M. melitensis* has not been isolated.

A chemical analysis of the milk was made by Dr. Micalef, with the following results:—

Total solids, 13·5 per cent.; fat, 4·1 per cent.; ash, 0·75 per cent.

Goat No. 16.—This goat was taken from the same herd as No. 15. On July 4 the blood was examined, and the serum diluted 1 to 60, was found to cause immediate clumping of the *M. melitensis.* The milk and urine have been examined daily since July 4, but up to the present the *M. melitensis* has not been isolated from either source.

Conclusions.—The results obtained show that some of the goats in every herd examined are suffering from Mediterranean Fever. The *M. melitensis* is exuded in the milk in enormous numbers when the disease has been present sufficiently long to cause a change in the physical characters of the fluid. It is also excreted in considerable numbers even when the animals are in "full milk," and no changes have occurred in either the physical or chemical characters of the milk.

The *M. melitensis* is also excreted in the urine of goats suffering from Mediterranean Fever, but up to the present it has only been found when the disease has existed for some time and physical changes have occurred in the milk.

VIII. EXAMINATION OF GOATS' BLOOD FOR REACTION TO MEDITERRANEAN FEVER.

By J. Crawford Kennedy, R.A.M.C., Member of the Mediterranean Fever Commission, Malta.

No. of herd.	Owner and number in each herd.	Address.	Milk supplied to—	Total number examined.	Number that gave no reaction.	Number that reacted to Med. Fever.	Percentage of reactions.	Table showing amount of reaction in each infected goat by dilutions up to $\frac{1}{100}$.						
								Dilution.	10.	20.	40.	60.	80.	100.
1	C—, Nos. 1 to 4 and 74 to 83	Casal Tarshiel near Casal Paulo	Cottonera Hospital, Zabbar Gate and near lying part of town	14	7	7	50	No. of goat	2 80	74 78	1 75	... ...	... ...	83 ...
								Total ...	2	2	2	...	...	1
2	A— M— and F— G—, Nos. 5 to 17	Zabbar ...	Cottonera Hospital, Zabbar Gate and near lying town	13	12	1	7·6	No. of goat	...	...	...	...	...	5
								Total ...	...	...	...	...	...	1
3	J—, Nos. 18 to 20	Hamrun ...	Valetta	3	3	...	...	...	...	...	...	...	...	...
4	J— F—, Nos. 21 to 23	Casal Curmi	Valetta Hospital and town	13	9	4	30·7	No. of goat	... ...	32 30	... ...	32 ...	... ...	27 ...
								Total ...	...	2	...	1	...	1

No. of herd	Owner and number in each herd.	Address.	Milk supplied to—	Total number examined.	Number that gave no reaction.	Number that reacted to Med. Fever.	Percentage of reactions.	Table showing amount of reaction in each infected goat by dilutions up to $\frac{1}{100}$.						
								Dilution.	10.	20.	40	60.	80.	100.
5	C—, Nos. 34 to 48	St. George's	Forrest Hospital ...	15	10	5	33·3	No. of goat	...	38	...	37	...	...
									...	39	...	...	...	...
									...	43	...	...	...	...
									...	48	...	...	...	...
								Total ...	...	4	...	1	...	...
6	M— M—, Nos. 49 to 73	St. John's Ditch	Valetta	25	8	17	68	No. of goat	51	54	...	64	...	50
									59	66	...	...	...	52
									62	67	...	...	...	55
									63	70	...	...	...	60
									65	...	...	...	...	68
									73	...	...	...	...	71
								Total ...	6	4	...	1	...	6
7	G— M—, Nos. 84 to 129	Hamrun ...	Valetta	46	20	26	56	No. of goat	97	88	85	103	...	99
									109	93	90	111	...	102
									117	96	...	119	...	105
									120	106	...	126	...	121
									123	110	...	...	...	122
									...	112	...	...	...	129
									...	113	...	...	...	...
									...	128	...	...	...	...
									...	128	...	...	...	...
								Total ...	5	9	2	4	...	6

8	F— M—, Nos. 130 to 161	Pietá ...	Valetta	32	8	24	75	No. of goat	137	134	131	147	...	132
									140	135	157	...	...	133
									144	141	...	...	...	136
									148	149	...	...	...	138
									...	...	...	...	...	145
									...	...	...	...	...	151
									...	...	...	...	...	153
									...	...	...	...	...	154
									...	...	...	...	...	155
									...	...	...	...	...	156
									...	...	...	...	...	159
									...	...	...	...	...	160
									...	...	...	...	...	161
								Total ...	4	4	2	1	...	13
			Total	161	77	84	52·17							

Examination of a Herd of Goats kept Privately and not allowed outside their own Field, as a Comparison with the Herds that Walk into Town Daily.

Total examined	10
No reaction	5
React to Mediterranean Fever	5

Of the five which reacted:—

1 reacted in dilution	$\frac{1}{10}$
3 " "	$\frac{1}{20}$
1 " "	$\frac{1}{100}$

∴ The percentage of infected goats in this herd is 50 per cent., comparing very closely with 52 per cent. of the public herds.

IX. RESULTS OF EXAMINATIONS FOR THE ISOLATION OF *MICROCOCCUS MELITENSIS* FROM THE BLOOD, URINE, AND SPUTUM OF CASES INFECTED WITH MEDITERRANEAN FEVER IN HASLAR HOSPITAL.

By P. W. BASSETT-SMITH, Fleet-Surgeon, Haslar.

Blood.—The blood was obtained from the median basilic vein of the arm, which had been carefully sterilised; from 1 to 3 c.c. were taken with an all-glass anti-toxin syringe; the blood was at once injected into flasks containing 50 c.c. of peptone broth; from this sub-cultures were made on to agar daily for 14 days at least, the resulting growth being tested by—

(1) Agglutination with specific serum.
(2) Alkaline reaction with litmus milk.
(3) Negative staining by Gram.

In all 27 bloods were examined from 24 patients, with 16 positive and 11 negative results, as shown in the following table :—

No.	Day of disease.	Condition.	Temperature.	Amount of blood taken.	Result.
			° F.	c.c.	
1	50	Acute relapse	102·4	3	Positive
2	142	Slight „	99·4	2·5	„
3	84	Acute „	102	3	„
4	117	„ „	103·4	3	„
5	92	„ „	102	3	„
6	23	Prim. wave	104	3	„
7	34	Sec. „	103	3	„
8	167	Relapse..........................	102	1	Negative
			short relapse with neuritis		
9	143	Acute relapse	101	2	Positive
10	44	„ „	101·2	2·5	„
11	105	„ „	102·6	3	„
12	153	„ „	105	2	„
13	44	„ „	102·8	0·5	„
14	41	„ „	102	2	„
15	80	„ „	102·6	2	„
16	111	„ „	101	2	„
17	61	„ „	103	2	„
18	122	Convalescent	N.	1	Negative
19	185	Cachexia.........................	N.	2	„
20	110	Slight relapse	100	3	„
			slight wave, not repeated		

No.	Day of disease.	Condition.	Temperature.	Amount of blood taken.	Result.
			° F.	c.c.	
21	165	Convalescent	N.	3	Negative
			went out next day		
22	159	,, ...	N.	3	Negative
			no return of the fever		
23	130	,, ...	N.	3	Negative
24	78	,, ...	N.	3	,,
			no return of the fever		
25	134	,, ...	N.	3	Negative
			no fever, great anæmia		
26	120	,, ...	99	3	Negative
			flask contaminated on the 14th day		
27	1 year	Cachexia	N.	3	Negative

Two flasks after being inoculated were found to be contaminated and are not included in this list, the others were either sterile or contained a pure culture of *M. melitensis.*

Excepting for cases 8, 20, and 26, the *M. melitensis* was recovered from all cases examined where fever was present. When not found I think the prognosis is favourable for continued convalescence.

Urines.—The technique as recommended by Major Horrocks was followed—the penis being washed with carbolic acid solution, the urine collected in a sterile test-tube after a part had been passed to clear the urethra, and then 0·2 c.c. plated on litmus-glucose-nutrose-agar, incubated at 37° C.

In all 46 urines have been thus examined, of 18 patients, with the following results. In the great majority of the plates, in 24 hours the surface was covered by a spreading foul-smelling acid organism, or was thickly studded with opaque rapidly growing colonies of a rather large coccus, but in two instances typical colonies of *M. melitensis* were present as a pure culture, both being from the same patient.

No. of case.	No. of examinations.	Condition.	Result.
1	1	Acute relapse	Negative
2	7	,, ,, ...	,,
3	1	,, ,, T. 103	,,
4	1	,, ,, ...	,,
5	2	,, ,, ...	,,
6	5	,, ,, ...	,,
7	3	,, ,, ...	,,
8	1	Convalescent	,,
9	7	,, ...	,,
10	2	Acute relapse	,,
11	2	,, ,, ...	,,
12	2	,, ,, ...	,,
13	1	,, ,, ...	,,
14	1	,, ,, ...	,,
15	4	,, ,, ...	,,
16	2	,, ,, ...	,,
17	11	,, ,, ...	1 with 16 col. on plate 1 with 14 col. on plate, pure culture of *M. melitensis* 9 rapidly overgrown
18	1	,, ,, ...	Negative

Unless the urine is free from other organisms, there seemed to be little chance of isolating the *M. melitensis*, the growth of this organism being so much slower than most of the others present.

Case 16 was very acute, in a typhoid condition, and the urine was drawn off with a catheter, but was full of other organisms.

Sputum.—As several of the cases recently received into Haslar were suffering from bronchial catarrh, with expectoration of mucoid sputum, I thought it possible that in these one might be able to isolate the organism from the sputum.

Technique.—The sputum in the early morning was received into a sterile test-tube, a fragment of the thickest portion was then fished out on a platinum loop, thoroughly washed in a tube of sterilised water, and again removed to a second test-tube of sterile water, thoroughly mixed, and 0·2 c.c. plated out on nutrose-glucose-litmus-agar, incubated at 37°.

So far no colonies resembling the *M. melitensis* have been met with, and as all the cases have ceased to expectorate, the experiment has ceased.

No. of case.	No. of experiment.	Condition.	Character of sputum.	Result.
1	1	Acute relapse	Muco. purt.	Negative
	2	,, ,, ...	,, ...	,,
2	3	Convalescent	Mucoid	,,
3	4	Acute relapse	,, ...	,,
4	5	,, ,, ...	,, ...	,,
	6	,, ,, ...	,, ...	,,
5	7	,, ,, ...	,, ...	,,
	8	,, ,, ...	,, ...	,,
	9	,, ,, ...	,, ...	,,
6	10	,, ,, T. 104......	,, ...	,,

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